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THE EFFECT OF THE SUBCUTANEOUS INJECTION OF ADRENALIN CHLORID ON THE HEAT PRODUCTION, BLOOD PRESSURE AND PULSE RATE IN MAN

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The results of previous studies of the effect on the respiratory exchange of the injection of adrenalin chlorid may be briefly summarized: An increase in the respiratory quotient was found by Fuchs and Roth (1), Hari (2), Bernstein and Falta (3), Lusk and Riche (4), (5) and by Tompkins, Sturgis and Wearn (6). La Franca (7), Wilenko (8) and Bernstein (9) found no change. A greater oxygen consumption or calorific output than normal was noted by La Franca (7), Fuchs and Roth (1), Bernstein and Falta (3), Bernstein (9), Lusk and Riche (4), (5), and by Tompkins, Sturgis and Wearn (6); Wilenko (8) found no change in the oxygen consumption and Hari (2) and Jackson (10) found a decrease.

Of these contributions the most important is that of Tompkins, Sturgis and Wearn (6). In a carefully controlled series of 34 experiments they obtained, without exception, an increase in the heat production following the injection of adrenalin chlorid (0.5 cc. of 1/1000) and this increase was usually accompanied by a rise in the respiratory quotient (27 experiments). Soldiers were studied; they were divided into three groups: *a*, Those with "irritable heart;" *b*, those with hyperthyroidism; and *c*, normal men, that is, well-trained active soldiers on full duty. There were 25 soldiers with "irritable heart," in 13 of whom the increase in the metabolism from the adrenalin injection was accompanied by an increase in the pulse rate and systolic blood pressure of at least ten points each, while in 12 there was a less marked increase. In the 3

TABLE 1

The effect of the subcutaneous injection of adrenalin chlorid on the blood pressure, pulse rate, respiration rate, ventilation rate, respiratory quotient, total calories per hour and basal metabolic rate (standard dose of adrenalin chlorid 0. 5 cc.)

GROUP	CASE	DIAGNOSIS	SEX	AGE	WEIGHT	HEIGHT	BLOOD PRESSURE					PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC RATE		RESPIRATION RATE		VENTILATION RATE		NOTES
							Basal		Highest																	
							Systolic	Diastolic	Systolic	Diastolic	Percentage increase over basal in systolic	Basal	Highest	Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Basal	Highest	Basal	Highest	Basal	Highest	
					kgm.	*cm.*																				
1	263084*	Exophthalmic goiter	M	28	57.6	176.4	154	70	165	70	7	121	128	6	137.6	143.3	4	0.75	0.80	+103	+111	23	25	10.06	11.14	Dosage of adrenalin less than standard
	247994*	Exophthalmic goiter	F	36	41.7	157.5	126	76	155	85	23	131	156	19	92.6	113.1	22	0.83	0.95	+85	+126	20	24	6.56	9.31	Dosage of adrenalin less than standard
	259130	Exophthalmic goiter	M	40	67.5	179.1	163	85	178	75	9	118	139	18	125.4	150.7	20	0.77	0.88	+76	+112	22	26	10.60	12.89	
	258885	Exophthalmic goiter	M	34	58.5	178.4	156	72	200	90	28	94	128	36	118.9	161.0	35	0.71	0.91	+74	+136	24	26	9.03	15.22	
	260316	Exophthalmic goiter	M	32	52.3	167.6	136	76	166	80	22	90	118	31	107.0	128.2	20	0.76	0.83	+71	+105	22	26	7.82	10.10	
	262154*	Exophthalmic goiter	F	37	41.4	164.2	136	68	140	70	3	106	135	27	85.3	101.1	19	0.75	0.90	+66	+96	17	26	6.48	8.88	Dosage of adrenalin less than standard
	262154*	Exophthalmic goiter			44.0		120	75	135	78	13	110	129	17	69.4	77.5	12	0.73	0.79	+31	+47	8	10	4.70	5.96	Dosage of adrenalin less than standard; 14 days after thyroidectomy
	258794	Exophthalmic goiter	F	51	43.4	156.9	130	70	155	70	19	123	141	15	77.4	95.3	23	0.75	0.85	+60	+97	16	28	5.71	7.24	
	260358	Exophthalmic goiter	F	26	47.5	162.6	130	64	146	80	12	112	138	23	86.6	97.5	13	0.78	0.88	+58	+78	20	24	6.14	7.94	

2	261849	Exophthalmic goiter	F	24	49.1	163.7	120	72	146	78	22	92	114	24	66.8	79.7	19	0.79	0.86	+20	+43	14	14	4.98	6.27	
	262089	Exophthalmic goiter	M	20	61.1	161.9	128	78	138	85	8	104	112	8	77.4	85.1	10	0.67	0.76	+18	+30	20	22	5.45	7.07	
	262089*	Exophthalmic goiter			59.3		120	78	141	77	18	104	112	8	75.6	90.8	20	0.72	0.82	+17	+40	22	26	5.84	7.39	Thyroxin 5 days previous to this test
	261192	Exophthalmic goiter	F	19	55.5	163.2	120	80	146	68	22	97	107	10	68.2	79.5	17	0.81	0.82	+13	+32	22	32	6.00	7.66	
	261192*	Exophthalmic goiter			55.4		128	80	154	86	20	105	135	29	73.8	88.2	20	0.84	0.96	+22	+46	28	34	7.50	9.38	Thyroxin 5 days previous to this test
	260662	Exophthalmic goiter	M	18	59.9	168.3	116	82	119	76	3	80	80	0	74.9	85.1	14	0.84	0.84	+9	+24	19	16	5.18	6.02	
3	245549	Adenomas of thyroid; hyperthyroidism	M	44	76.1	163.2	135	80	148	82	10	84	144	71	83.9	103.4	23	0.84	0.96	+20	+48	17	16	6.40	8.32	
	245549*	Adenomas of thyroid; hyperthyroidism			74.4		136	65	154	66	13	96	116	21	81.1	97.9	21	0.80	0.93	+16	+41	18	22	5.79	7.98	Thyroxin 7 days previous to this test
	261171	Adenomas of thyroid; hyperthyroidism	F	59	60.5	161.3	152	92	158	76	4	63	79	25	65.3	79.2	21	0.89	0.92	+14	+38	18	19	6.67	7.94	
	262353	Adenomas of thyroid; hyperthyroidism	M	48	73.0	176.5	125	75	145	75	16	86	116	35	82.0	103.9	27	0.77	0.98	+12	+42	15	24	5.67	9.67	

TABLE 1—*Continued*

GROUP	CASE	DIAGNOSIS	SEX	AGE	WEIGHT	HEIGHT	BLOOD PRESSURE					PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC RATE		RESPIRATION RATE		VENTILATION RATE		NOTES
							Basal		Highest																	
							Systolic	Diastolic	Systolic	Diastolic	Percentage increase over basal in systolic	Basal	Highest	Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Basal	Highest	Basal	Highest	Basal	Highest	
					kgm.	*cm.*																				
4	119415	Double oöphorectomized neurasthenic; hyperthyroidism	F	21	45.5	164.4	114	74	130	70	14	74	112	51	62.7	83.2	33	1.06	1.17	+15	+53	16	22	6.72	12.10	
5	287796	Adenomas of thyroid without hyperthyroidism	F	30	59.5	174.9	125	75	118	74	−6	82	104	27	65.4	72.3	11	0.71	0.77	+4	+15	18	18	5.57	6.64	
6	256032	Colloid goiter; hypothyroidism	F	17	47.2	153.7	116	70	132	80	14	62	78	26	48.0	61.6	28	0.90	0.88	−16	+9	15	20	3.69	5.20	
7	262125	Addison's disease?	F	32	53.7	170.2	98	70	104	80	6	100	95	−5	57.3	66.3	16	0.82	0.80	−3	+12	14	16	5.34	5.63	
	261437	Addison's disease?	F	35	32.2	158.1	80	58	86	52	8	94	99	5	41.3	47.9	16	0.83	0.80	−8	+7	17	17	4.20	5.01	

8	285194	Hypopituitarism	M	33	79.5	193.4	122	86	132	90	8	64	91	42	77.7	93.2	20	0.77	0.97	−6	+13	8	13	5.05	6.99	
	261342	Hypopituitarism	M	33	67.4	177.8	90	64	96	62	7	62	68	10	63.4	72.0	14	0.72	0.77	−13	−1	16	18	4.58	6.17	
	261342*	Hypopituitarism			64.7		116	78	118	66	2	58	90	55	68.2	82.9	22	0.65	0.85	−5	+16	16	18	4.20	7.00	Thyroxin 6 days previous to this test
	276369	Hypopituitarism	M	44	62.8	173.2	90	60	88	64	−2	57	64	12	48.0	52.2	9	0.82	1.04	−28	−22	12	18	4.61	6.61	
9	262555	Myxedema	M	39	64.0	179.7	114	88	122	70	7	60	59	−2	42.9	47.2	10	0.83	0.85	−40	−34	10	13	3.25	3.66	
	262555*	Myxedema			59.2		128	80	160	90	25	64	79	23	64.4	79.2	23	0.79	0.88	−7	+15	16	18	5.06	6.23	Thyroxin 7 days previous to this test
	262235	Myxedema	F	49	68.6	160.6	120	90	132	85	10	73	76	4	43.5	48.0	10	0.82	0.81	−30	−23	10	9	2.74	3.06	
	259013*	Myxedema	F	46	84.7	155.6	104	68	124	66	19	87	98	13	65.8	78.5	19	0.74	0.82	−1	+19	22	30	5.90	8.05	Thyroxin 19 days previous to this test
10	61699	Postoperative myxedema	F	41	54.1	163.2	130	86	112	80	−14	70	76	9	49.5	60.5	22	0.93	1.06	−13	+7	17	9	3.91	5.19	
	137062	Postoperative myxedema	F	37	74.5	162.6	118	90	135	90	14	77	88	14	54.2	73.7	36	0.79	0.85	−18	+12	17	19	4.33	6.52	
11	257217	Atypical hypothyroidism on thyroxin	M	56	52.3	173.4	122	68	132	68	8	65	96	48	66.7	94.5	42	0.78	0.89	+10	+55	14	17	4.73	6.58	
12	276977	Anorexia nervosa; hypothyroidism	F	16	37.8	170.6	80	58	104	52	30	79	84	6	43.5	46.8	8	0.84	0.85	−22	−16	18	20	4.02	4.61	

TABLE 1—*Concluded*

GROUP	CASE	DIAGNOSIS	SEX	AGE	WEIGHT	HEIGHT	BLOOD PRESSURE					PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC RATE		RESPIRATION RATE		VENTILATION RATE		NOTES
							Basal		Highest																	
							Systolic	Diastolic	Systolic	Diastolic	Percentage increase over basal in systolic	Basal	Highest	Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Basal	Highest	Basal	Highest	Basal	Highest	
					kgm.	*cm.*																				
13	261292	Neurasthenia	M	23	77.1	177.1	120	80	140	80	17	83	95	14	79.8	100.3	26	0.77	0.80	+4	+30	9	11	4.81	6.37	
	261292*	Neurasthenia			76.8		138	76	133	80	−4	99	108	9	97.7	116.4	19	0.80	0.87	+28	+52	12	10	5.74	7.70	Thyroxin 5 days previous to this test
	280559	Neurasthenia	F	26	46.3	151.3	106	76	120	68	13	88	104	18	56.3	62.3	11	0.71	0.77	+9	+21	18	20	4 42	5.15	
	257871	Neurasthenia	F	23	72.6	160.6	110	85	118	80	7	89	96	8	65.3	72.2	11	0.83	0.86	+1	+11	15	19	5.04	6.01	
	281027	Chronic nervous exhaustion	F	21	52.0	169.5	104	68	112	64	8	78	96	23	55.8	64.7	16	0.75	0.84	−5	+10	19	24	4.63	6.19	
	281509	Chronic nervous exhaustion	F	32	42.2	153.1	108	68	140	74	3	83	97	17	58.6	86.9	48	0.83	0.98	+20	+78	21	39	5.16	12.76	
14	165334	Normal control	M	22	70.5	174.7	118	78	142	78	20	77	104	35	79.7	99.3	25	0.73	0.89	+9	+36	12	18	4.95	7.44	
	263723	Normal control	M	47	68.8	175.0	128	80	140	70	9	67	84	25	79.5	88.7	12	0.82	0.81	+12	+25	18	17	6.20	6.74	
	263068	Normal control	M	37	67.8	172.9	130	82	140	70	8	84	88	5	70.0	85.3	22	0.90	0.83	−2	+19	19	16	7.11	8.69	
	263661	Normal control	M	25	70.5	178.3	104	65	124	74	19	60	73	22	79.3	94.6	19	0.77	0.82	+7	+27	16	21	5.44	7.69	

* The cases starred are omitted from the group averages given in Table 2.

cases of hyperthyroidism there was an increase of more than ten points in both the pulse and systolic blood pressure, while in the 6 normal soldiers on active duty the response in the pulse rate and systolic blood pressure was less definite.

Before the publication of the paper by Tompkins, Sturgis and Wearn, an investigation on the effect of the subcutaneous injection of adrenalin on the heat production, pulse rate, and blood pressure had been started in this laboratory; a preliminary report has recently been made (11) of the results given here in detail. The method of procedure was as follows: The patients came to the laboratory in the so-called post-absorptive condition and the basal metabolic rate, pulse rate, and blood pressure were determined after the patients had rested in bed twenty minutes according to the regular routine practiced in this laboratory and described in detail elsewhere (12). For the determination of the basal metabolic rate a mask is tightly adjusted over the patient's mouth and nose and, by means of expiratory and inspiratory valves, the total volume of the patient's expired air is collected in a gasometer for a known period of approximately ten minutes. Duplicate determinations are made of the carbon dioxid and oxygen content of the expired air, the analyses being done in the Haldane gas analysis apparatus. From the data obtained the basal metabolic rate is calculated by the usual method.

After the various basal values have been determined, 0.5 cc. of 1/1000 solution of adrenalin chlorid (Parke, Davis & Company) is injected subcutaneously in the patient's arm; the pulse and blood pressure are taken every five minutes for the next two hours and the metabolic rate determined for approximately ten-minute periods beginning about ten minutes, thirty minutes, one hour, and one and one-half hours after the injection.

Forty-six experiments were done on the 39 patients grouped according to diagnosis in table 1. The results of these experiments are summarized in table 2. Four experiments in group 1 are not included in the average given in table 2 because the dose of adrenalin was less than 0.5 cc.; 2 experiments in group 2, 1 in group 3, 1 in group 8, 2 in group 9 and 1 in group 13 are not included in the average because of previous thyroxin administration. The experiments omitted from the average are starred in table 1. The data obtained as basal before the adrenalin injection are contrasted in table 1 with the data which showed the most pronounced reaction following the injection. The percentage increase over the basal is given for the total calories, pulse rate, and systolic blood pressure. In table 2 are given the average data for the

various groups of cases studied. In table 3 are grouped those cases in which the adrenalin test was done both before and after the patients were given thyroxin.

TABLE 2

Summary of the effect of the subcutaneous injection of adrenalin chlorid on the systolic blood pressure, pulse rate, respiration rate, ventilation rate, respiratory quotient, total calories per hour and basal metabolic rate (standard dose of adrenalin chlorid 0.5 cc.)

GROUP	NUMBER OF CASES	DIAGNOSIS	AVERAGE B. M. R. FOR EACH GROUP	PERCENTAGE VARIATIONS FROM BASAL			NUMERICAL VARIATIONS FROM BASAL			
				Systolic blood pressure	Pulse rate	Total calories per hour	Respiration rate	Ventilation rate: liters S. T. P. D.	Respiratory quotient	Metabolic rate
			per cent	*per cent*	*per cent*	*per cent*				
1	9	Exophthalmic goiters; five with B. M. R. above +50 per cent	+68	18	25	22	5	2.82	0.12	38
2		Four with B. M. R. below +50 per cent	+15	14	11	15	2	1.35	0.04	17
		Average of all exophthalmic goiters	+44	16	18	19	4	2.17	0.08	29
3	3	Adenomas of thyroid; hyperthyroidism	+15	10	44	24	3	2.40	0.12	27
4	1	Double oöphorectomized neurasthenic with hyperthyroidism	+15	14	51	33	6	5.38	0.11	38
5	1	Adenoma of thyroid without hyperthyroidism	+4	−6	27	11	0	1.07	0.06	11
6	1	Colloid goiter; hypothyroidism	−16	14	26	28	5	1.51	−0.02	25
7	2	Addison's disease	−6	7	0	16	1	0.55	−0.03	15
8	3	Hypopituitarism	−16	4	21	14	4	1.84	0.16	12
9	2	Myxedema	−35	9	1	10	1	0.37	0.01	7
10	2	Postoperative myxedema	−16	0	12	29	−3	1.74	0.10	25
11	1	Atypical hypothyroidism on thyroxin	+10	8	48	42	3	1.85	0.11	45
12	1	Anorexia nervosa; hypothyroidism	−22	30	6	8	2	0.59	0.01	6
13	5	Neurasthenia and chronic nervous exhaustion	+6	10	16	22	6	2.48	0.07	24
14	4	Normal clinical controls	+7	14	22	20	2	1.72	0.03	20

DISCUSSION OF RESULTS

Heat production. In every experiment there was an increase in the heat production after the subcutaneous injection of adrenalin chlorid, the maximum occurring as a rule within ten to thirty minutes after the

injection. With but three exceptions, one patient with severe exophthalmic goiter in whom only 0.1 cc. adrenalin was injected, one patient with hypopituitarism, and one patient with anorexia nervosa, this maximum increase was at least 10 per cent above the basal; the greatest reaction was 48 per cent above the basal and occurred in a case of chronic nervous exhaustion. Within one and one-half to two hours after the adrenalin was injected the metabolic rate had returned to within 4 points of the preliminary level in 21 experiments; it was still somewhat elevated in 19 experiments, and it was slightly lower than the basal

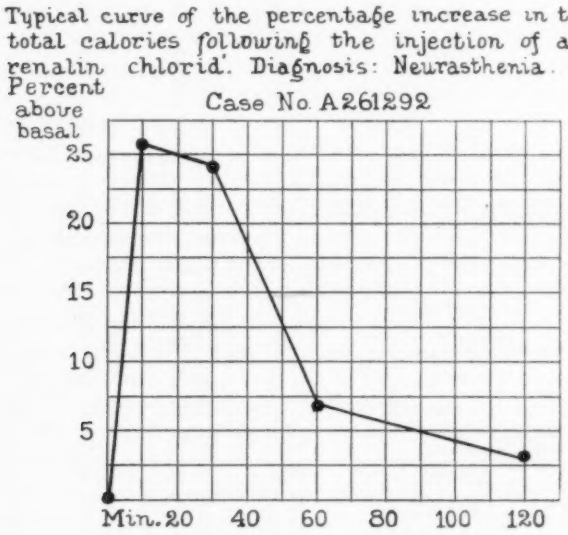

FIG. 1

determined before the injection in the remaining 6 experiments. A typical example of the post-adrenalin metabolic curve is given in figure 1.

In the group of patients with exophthalmic goiter with basal metabolic rates above +50 per cent who were given the standard dose of adrenalin the total number of calories per hour increased on the average 22 per cent over the basal, while in the group of patients with a milder degree of exophthalmic goiter whose basal metabolic rates were less than +50 per cent the total number of calories increased on the average 15 per cent. In spite of this no consistent relationship can be seen in the

TABLE 3

The effect of the subcutaneous injection of adrenalin chlorid, both before and after the intravenous injection of thyroxin, on the blood pressure, pulse rate, respiration rate, ventilation rate, respiratory quotient, total calories per hour and basal metabolic rate (standard dose of adrenalin chlorid 0.5 cc.)

CASE	DIAGNOSIS	BLOOD PRESSURE					PULSE RATE			TOTAL CALORIES PER HOUR			R. Q.		METABOLIC RATE		RESPIRATION RATE		VENTILATION RATE LITERS—S. T. P. D.		NOTES
		Basal		Highest																	
		Systolic	Diastolic	Systolic	Diastolic	Percentage increase over basal in systolic	Basal	Highest	Percentage increase over basal	Basal	Highest	Percentage increase over basal	Basal	Highest	Basal	Highest	Basal	Highest	Basal	Highest	
262089	Exophthalmic goiter...	128	78	138	85	8	104	112	8	77.4	85.1	10	0.67	0.76	+18	+30	20	22	5.45	7.07	
		120	78	141	77	18	104	112	8	75.6	90.8	20	0.72	0.82	+17	+40	22	26	5.84	7.39	8 mgm. thyroxin 5 days previous to test
261192	Exophthalmic goiter..	120	80	146	68	22	97	107	10	68.2	79.5	17	0.81	0.82	+13	+32	22	32	6.00	7.66	
		128	80	154	86	20	105	135	29	73.8	88.2	20	0.84	0.96	+22	+46	28	34	7.50	9.38	8 mgm. thyroxin 5 days previous to test
245549	Adenomas of thyroid; hyperthyroidism....	135	80	148	82	10	84	144	71	83.9	103.4	23	0.84	0.96	+20	+48	17	16	6.40	8.32	
		136	65	154	66	13	96	116	21	81.1	97.9	21	0.80	0.93	+16	+41	18	22	5.79	7.98	8 mgm. thyroxin 7 days previous to test
261342	Hypopituitarism.....	90	64	96	62	7	62	68	10	63.4	72.0	14	0.72	0.77	−13	−1	16	18	4.58	6.17	
		116	78	118	66	2	58	90	55	68.2	82.9	22	0.65	0.85	−5	+16	16	18	4.20	7.00	5 mgm. thyroxin 6 days previous to test
262555	Myxedema.....	114	88	122	70	7	60	59	−2	42.9	47.2	10	0.83	0.85	−40	−34	10	13	3.25	3.66	
		128	80	160	90	25	64	79	23	64.4	79.2	23	0.79	0.88	−7	+15	16	18	5.06	6.23	5 mgm. thyroxin 7 days previous to test
261292	Neurasthenia.....	120	80	140	80	17	83	95	14	79.8	100.3	26	0.77	0.80	+4	+30	9	11	4.81	6.37	
		138	76	133	80	−4	99	108	9	97.7	116.4	19	0.80	0.87	+28	+52	12	10	5.74	7.70	5 mgm. thyroxin 5 days previous to test

various groups studied and summarized in table 2, between the height of the adrenalin reaction and the degree of hyperthyroidism or hypothyroidism as determined by the level of the metabolic rate. This inconsistency is further shown in the 6 cases, (table 3) in which the adrenalin reaction was studied both before and after the intravenous injection of thyroxin for in two of these the percentage increase in the total calories after the adrenalin injection was less instead of greater in the adrenalin test carried out after the patients had received thyroxin. Furthermore, there is only a very slight difference in the average increase in the heat production from the adrenalin injection before thyroxin was administered (17 per cent) as compared with the average increase after thyroxin was given (21 per cent).

The metabolic curve resulting from the subcutaneous injection of adrenalin is entirely different from that found by Plummer following the intravenous injection of 16 mg. of thyroxin. Following the injection of adrenalin the height of the metabolic curve is reached in approximately from ten to thirty minutes and has returned to its basal level in about two hours; when thyroxin is injected, however, the height is not reached for approximately from three to ten days and the basal level not regained for from one to two months. The presence of hyperglycemia (13) following the injection of adrenalin chlorid naturally directs attention to the similarity of the post-adrenalin metabolic curve to that found by Lusk (14) as a result of carbohydrate plethora following the ingestion of glucose.

Ventilation and respiration rates. There is invariably an increase in the ventilation rate following the injection of adrenalin chlorid and this is usually accompanied by an increase in the respiration rate, although there may even be a decrease in the latter. The variations in the percentages of carbon dioxid eliminated in the expired air and of oxygen absorbed from the inspired air vary inversely with the changes in the ventilation rate and directly with the increase in the metabolism. It is impossible, however, to predict the degree with which the increase in the respiratory volume will predominate over the concentration of that volume in different individuals or even in the same individual at different times.

Respiratory quotient. Following the injection of adrenalin there was an increase in the respiratory quotient in 39 experiments, no change in 1 experiment, and a decrease in 6. These results taken in conjunction with those of Tompkins, Sturgis and Wearn (6) indicate that as a rule there is an increase in carbohydrate combustion; this is confirmed by

the findings of other observers of an increase in the blood sugar (13) following the injection of adrenalin. The action of adrenalin, therefore, is not only to cause a higher rate of oxidation but also to mobilize carbohydrate as fuel for this increased combustion; which reaction is primary is not known.

Diastolic blood pressure. Throughout the groups there is no constant variation in the diastolic blood pressure. In 19 experiments there was an increase, in 7 there was no change, and in 20 there was a decrease. If the peripheral capillary cross section remains constant when the blood flow is increased the diastolic blood pressure necessarily rises; an unchanged or decreased diastolic with increased blood flow would indicate a compensatory peripheral dilatation. Even a certain proportion of those experiments that showed a slight increase in diastolic blood pressure may likewise have had a slight peripheral dilatation that was not, however, of sufficient degree entirely to compensate for the increased blood flow. A peripheral compensatory dilatation is, therefore, indicated by the data in 27 experiments; it may have occurred in an unknown proportion of the other 19 experiments. Further evidence of peripheral dilatation is shown by the flushing of the skin and increased perspiration, which are physical compensatory factors to accommodate the body to the increased production of heat.

Systolic blood pressure. An increase in the systolic blood pressure was noted in all but 4 cases following the adrenalin injection. These four are as follows: One case of adenomas of the thyroid without hyperthyroidism with a basal metabolic rate of +4 per cent; one case of hypopituitarism with a basal metabolic rate of −28 per cent; one case of post-operative myxedema with a basal metabolic rate of −13 per cent; and one case of neurasthenia with a rate of +28 per cent after an injection of thyroxin. In 18 cases the increase was less than 10 per cent; in 17 the increase was between 10 per cent and 20 per cent, and in 7 the increase was 21 per cent or more above the basal. From a study of the individual experiments, of the averages of the various groups of patients, or of the experiments carried out after the patients had received thyroxin, no consistent parallelism can be seen between the percentage increase in the systolic blood pressure following the injection of adrenalin and the degree of over- or under-activity of the thyroid gland.

Pulse rate. The pulse rate increased in all but 3 cases: One of these was a case of myxedema in which the basal metabolic rate was − 40 per cent; the second, a case of Addison's disease in which the basal

metabolic rate was −3 per cent; and the third, a case of mild exophthalmic goiter in a stage of remission in which the basal metabolic rate was + 9 per cent. Ten cases showed less than a 10 per cent increase; all the rest showed a greater response. With an increase in the oxygen consumed and carbon dioxid produced following the injection of adrenalin the circulatory system must transport larger amounts of these and of other substances. This can be accomplished in two ways: *a*, by an increase (15), (16) in the blood flow which can be brought about either by an increase in the number of beats of the heart for each minute, or by an increase in the volume of each beat (or by both, or by a large increase of one with a decrease of the other); and *b*, by a unit volume

TABLE 4

Summary of effect of subcutaneous injection of adrenalin chlorid on blood pressure, pulse rate, and respiration rate (standard dose of adrenalin chlorid 0.5 cc.)

NUMBER OF CASES	DIAGNOSIS	AVERAGE B. M. R.	PERCENTAGE INCREASE OVER BASAL	
			Systolic blood pressure	Pulse
		per cent	*per cent*	*per cent*
8	Exophthalmic goiter	+32	14	18
6	Adenomas of thyroid with hyperthyroidism	+30	17	11
4	Adenomas of thyroid without hyperthyroidism	−1	7	14
2	Colloid goiter	+3	3	17
4	Cardiac disease	+2	13	25
3	Neurasthenia	+4	6	15
1	Malignancy	−3	3	21
1	Pulmonary tuberculosis	+20	18	6

of blood carrying a greater load. Which of these variable factors will predominate is of course impossible to predict. Furthermore, no consistent relationship can be seen following the injection of adrenalin between the response of the pulse rate and the degree of activity of the thyroid gland.

Supplementary experiments. A second series of 29 experiments was carried out in a manner similar to that employed in the preceding series except that the metabolic rates were not determined after the administration of adrenalin. The results are presented in a summarized form in table 4. As in the first series, there is in the various groups an average irregular increase in both pulse rate and systolic blood

pressures. We can discern no parallelism between the changes in pulse rate and blood pressures and the degree of hyperthyroidism that would in any way render the reaction of diagnostic value in such conditions, as has been suggested by Goetsch (17).

GENERAL DISCUSSION

These experiments indicate that the injection of adrenalin chlorid produces invariably an increase in the rate of cellular combustion varying between a calorific increase of from 4 per cent to 48 per cent. This increase is accompanied as a rule by an increase in the ventilation rate, respiration rate, number of heart beats for each minute, volume of each beat, greater utilization of the blood-carrying power and peripheral dilatation with an increased systolic and decreased diastolic blood pressure. Not all these compensatory factors necessarily come into play in each instance; as would be expected, various combinations may occur, sometimes one factor, sometimes another factor acting as the major compensation. In individual instances it is impossible to predict the combination, although in perfectly healthy and well-trained persons such as those in the group of normal soldiers studied by Tompkins, Sturgis and Wearn, each compensation factor plays its rôle so well and so easily that there is discernible only the slightest increase of any one factor, while in the case of ill-acting hearts (irritable hearts) the response to extra demands is not smoothly and efficiently accomplished. This is true also in any condition like hyperthyroidism in which the circulatory system is more or less damaged and already severely taxed by its own increased metabolism, and as a result an additional load is not readily borne.

No relationship was found in our experiments between the intensity of the reaction and the degree of hyper- or hypothyroidism. There is no sound physiologic foundation, so far as we can see, for the assumption that the character of the reaction following the injection of adrenalin chlorid is indicative of the activity of the thyroid gland.

The cause of the increased heat production is unknown. The similarity of the metabolic rate curve following the injection of adrenalin to that found by Lusk from a carbohydrate plethora naturally directs attention to the possibility that the increased heat production is due to an excess of carbohydrate metabolites. In addition there may be, however, a direct chemical stimulation of cellular combustion. In either case the phenomenon is obviously in harmony with Cannon's (18) "emergency theory" of the action of adrenalin.

SUMMARY

1. Forty-six experiments are reported on the effect of the subcutaneous injection of adrenalin chlorid on the metabolic rate, pulse rate, and blood pressure of patients suffering from various disorders of the ductless glands.

2. A supplementary series of 27 experiments is added in which a study was made of the effect of the adrenalin injection on the pulse rate, and blood pressure (the basal metabolic rate being known).

3. Adrenalin chlorid (0.5 cc. of 1 1000) injected subcutaneously invariably causes an increase in the metabolic rate. This increase is usually accompanied by an increase in the ventilation rate, respiration rate, number of heart beats each minute, volume of each beat, greater utilization of the blood carrying power and peripheral dilatation with an increased systolic and decreased diastolic blood pressure.

4. No relationship was found between the intensity of the adrenalin reaction and the degree of hyperthyroidism or hypothyroidism.

5. Attention is directed to the similarity of the metabolic rate curve following the injection of adrenalin to that found by Lusk from a carbohydrate plethora and to the possibility that the increased heat production is due to an excess of carbohydrate metabolites. It is suggested that in addition there may be a direct stimulation of cellular combustion.

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THE APPARENT INFLUENCE OF A DIET OF CARBOHYDRATES ON THE PANCREAS REMNANT OF PARTIALLY PANCREATECTOMIZED DOGS

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The experiments here reported were undertaken with the view of determining more definitely whether a carbohydrate diet prevents pancreatic hypertrophy or induces pancreatic degeneration, after partial pancreatectomy. The work was begun in 1916 and the experimental part brought to conclusion in 1917.

The question whether a diet rich in carbohydrates is the etiologic factor in those cases of human diabetes in which impairment of the pancreas cannot be related to known disease processes (cancer, infection, etc.) was put to experimental test in 1912 by Thiroloix and Jacob (1). These investigators reported that partially pancreatectomized dogs, remaining free from diabetes on a diet of meat, develop a fatal diabetes on prolonged carbohydrate feeding. They concluded that the pancreas remnant, adequate to prevent diabetes on a meat diet, is "overstrained" by the prolonged carbohydrate diet. As already noted by Allen (2), these investigators reported no "controls," showing that dogs with equally small pancreas remnants and on a meat diet will not show a similar tendency to gradual atrophy of the pancreas with attendant fatal diabetes. Such controls are necessary, as all workers in this field are agreed that even with the most careful estimate of the mass of pancreas tissue left in situ to just avoid permanent and fatal diabetes, one cannot predict whether the pancreas remnant will undergo hypertrophy so as to render the dog non-diabetic even on a carbohydrate diet, or whether it will atrophy so that the dog becomes permanently diabetic even on a protein diet.

In 1913 Allen (2) reported, in some detail experiments on eight dogs: "None of these observations were as long or as positive as those described by Thiroloix and Jacob. The impression gained, however, is in favor of the correctness of their position, viz., that the condition in

these dogs is aggravated by the carbohydrate diet" (p. 585). "Dogs at the outset diabetic (diabetes levis), yet they recover in spite of the heaviest carbohydrate diet and the most intense glycosuria" (p. 592).

In 1914 Allen (3) reported more positive conclusions, to the effect that dogs with partial pancreatectomy remaining permanently free from diabetes on meat are rendered permanently diabetic on a diet of bread, or bread plus glucose. It is also stated that in such diabetic dogs the island tissue undergoes "hydropic degeneration," similar to that noted by Homans.

In subsequent publications Allen (4) refers frequently to rendering partially depancreatectomized dogs permanently diabetic by a liberal diet of any of the three classes of foodstuffs (proteins, fats, or carbohydrates). The details of these experiments have, to our knowledge, not been published. It is expressly stated by Allen that fat in the diet brings on diabetes in dogs predisposed to diabetes by partial pancreatectomy, and that the prevention of diabetes in dogs with small pancreas remnants is not accomplished by withholding starches from the diet but by total reduction in the diet amounting to partial starvation. Allen's work on experimental diabetes in dogs has thus been gradually brought in accord with the starvation treatment of human diabetes, as inaugurated by Naunyn, and developed so brilliantly in this country by Allen.

Allen speaks of the hydropic degeneration of island tissue noted after partial pancreatectomy as a *result* rather than the cause of the diabetes —a result of "overstrain" on the endocrine function by a too liberal diet, and support for this "overstrain" hypothesis is sought by analogy in the evil effects of a liberal diet on persons with weakened digestive powers.

METHODS

In the preparation of the dogs great care was taken that the following conditions were established: *a*, that the pancreas remnant was connected with the duodenum through the main pancreatic duct, (D. Santorini), and that this duct remained patent; *b*, that each animal following the partial pancreatectomy showed no glycosuria on a liberal protein diet, but showed persistent glycosuria on a carbohydrate diet. Animals meeting these conditions were deemed serviceable for the investigation of this problem.

In the pancreatectomy operation the spleno-gastric and the pyloric portions of the pancreas were freed up to the D. Santorini, ligated off

and removed. Small fragments of pancreas tissue adhering to the duodenum were carefully removed. The pancreas remnant giving the desired glycosuria was assumed to be a fraction of approximately one-seventh of the total pancreas weight. This fraction was determined as follows: A piece of the excised pancreas was trimmed down to match that remaining attached to the duodenum. The weight of this piece added to the weight of all the pancreas tissue removed gives the total pancreas weight. The weight of the fraction of the pancreas remaining on the duodenum was determined by calculation, using the weights thus obtained. If this remnant was in excess of one-seventh of the total weight of the pancreas, the excess was ligated off and removed. Ligation was chosen to prevent hemorrhage of the cut ends, as well as to avoid fat necrosis.

The animals were fed once a day on an excess of one of the diets mentioned below. By an excess of diet is meant a quantity of food so large that some of the food was found in the pan on the following day at the time of the next feeding.

Three types of diets were used, designated as A, B and C.

Diet A. This consisted of a mixture of boiled starch, fresh bread and meat. The meat was either lung, liver or skeletal muscle (round steak). This diet contained approximately 60 per cent of carbohydrates.

Diet B consisted of a mixture of (equal parts) fresh bread and fresh meat (liver, lung or skeletal muscle). This is a low carbohydrate diet.

Diet C consisted of raw or boiled meat (skeletal muscle), depending on the preference of the individual animal. Diet *C* is therefore essentially a protein diet.

Diet *A* could not be fed continually over any long period of time, partly because it seemed to favor constipation, partly because, sooner or later, the dogs refuse to eat it. The two diets, *A* and *B*, were therefore used interchangeably. When the animals refused diet *A*, or alimentary stasis resulted because of constipation, they were given diet *B* and later put back on diet *A*. The diet used for determining the serviceability of the animals at the beginning of the experiment and to determine the degree of glycosuria in the later stages, was diet *B*, because as this contained a greater amount of meat it was ingested in greater quantities.

The animals were supplied with water and meat-free bones ad libitum. Twenty-four-hour samples of urine were collected from the dogs kept in metabolism cages for this purpose. Thymol was added to the urine as a preservative. The urine was analyzed for sugar by Benedict's method.

RESULTS

Dog A. Removed 19.5 grams of the pancreas October 17, 1916. Weight of pancreas remnant estimated 3.5 grams. Weight of dog at time of operation 10.2 kilos.

October 18 to November 6, 1916. Dog on meat diet. No polyuria. A trace of sugar in the urine on October 23, and October 26; urine free from sugar during the rest of the period.

November 7, 1916 to March 7, 1917. *Dog on diet B.* Slight polyuria (average 250 cc. per day). Sugar in the urine every day (0.21 per cent to 0.90 per cent). Weight of dog on March 7, 8.8 kilos.

March 7 to May 22, 1917. Dog on meat diet. Urine sugar-free. Weight of dog on May 22, 10.6 kilos.

May 23 to 26, 1917. *Dog on diet A.* No sugar in the urine. Laparotomy performed and 2.2 grams of the pancreas remnant extirpated. The pancreas remnant appeared hypertrophied.

May 27 to June 17, 1917. *Dog on meat diet;* slight polyuria (about 275 cc. per day) but no sugar in the urine. Weight of dog on June 17, 1917, 10.7 kilos.

June 18 to October 11, 1917. *Dog on diet A.* Marked polyuria (maximum 2300 cc. per day); gradual loss of weight and persistent glycosuria (2–7 per cent). Weight on October 11, 6.4 kilos. Dog posted. Weight of pancreas remnant, 7.4 grams. Dog extremely emaciated, skin ulcers, conjunctivitis.

This dog died in diabetes, despite the marked hypertrophy of the pancreas remnant. The dog did not tolerate the high carbohydrate diet very well, especially during the month of August, when severe gastro-intestinal disorders (diarrhea, vomiting, anorexia) developed.

Dog B. Removed 20.1 grams of the pancreas October 31, 1916. Pancreas remnant estimated at 3.1 grams. Weight of dog 8.5 kilos.

November 1 to 18, 1916. *Dog on meat diet.* Urine sugar-free.

November 19 to December 4, 1916. *Dog on diet B.* No polyuria, but persistent glycosuria (1–3 grams per day).

December 5, 1916 to January 8, 1917. *Dog on diet A.* Polyuria with persistent glycosuria (1–5 grams per day). Weight of dog January 8, 8 kilos.

January 9 to March 7, 1917. *Dog on diet B.* No polyuria, but slight glycosuria (0.5–1.0 gram per day).

March 8 to May 15, 1917. *Dog on meat diet.* No glycosuria. Weight of dog on May 15, 10.6 kilos.

May 16 to June 4, 1917. *Dog on diet A.* Slight polyuria, but no glycosuria. Weight of dog June 4, 8.5 kilos.

Laparotomy performed and 2 grams of the pancreas remnant removed. Remnant appeared hypertrophied.

June 5 to 18, 1917. *Dog on meat diet.* Slight polyuria, but no glycosuria. Weight of dog June 18, 9.6 kilos.

June 18 to July 22, 1917. *Dog on diet A.* Marked polyuria (maximum 2100 cc. per day) and persistent glycosuria of gradually increasing intensity (maximum 50 grams per day).

July 23 to 28, 1917. *Dog on meat diet.* Reduction in the polyuria (to about one-third) and reduction in the glycosuria (to about one-fourth). Weight of dog on July 27, 9.2 kilos.

July 27 to September 30, 1917. *Dog on diet B.* Persistent polyuria and glycosuria, with gradual loss of weight. Death September 30. Body weight 5.6 kilos. Pancreas remnant 5.4 grams. Dog extremely emaciated, with skin ulcers and conjunctivitis.

This dog died in typical diabetes gravis, despite the hypertrophy of the pancreatic remnant. Marked gastro-intestinal disorders developed in July, and were overcome by the meat diet period.

Dog C. Removed 15.5 grams pancreas June 14, 1917. Pancreas remnant estimated at 2.3 grams. Weight of dog 7.5 kilos.

June 15 to 21, 1917. *Dog on meat diet.* No glycosuria.

June 22 to August 9, 1917. *Diets A and B.* Polyuria (maximum 1200 cc. per day), glycosuria (maximum 30 grams per day) and progressive emaciation. Death. Weight at death, 5.4 kilos. *Pancreas remnant*, 2 grams Pancreatic duct patent. Dog had skin lesions and conjunctivitis.

Dog D. Weight 5 kilos; 8 grams of pancreas removed June 5, 1917. Pancreas remnant estimated 1.5 grams. Dog had enlarged thyroid.

June 5 to 24, 1917. *Meat diet.* Trace of sugar in urine, June 6 to 10. Urine sugar-free June 11 to 24.

June 25 to August 6, 1917. *Diet A.* Polyuria (maximum 880 cc. per day) and glycosuria (maximum 35 grams per day), and gradual loss of weight.

August 7 to 9, 1917. Starvation. Urine sugar-free August 8 and 9.

August 10 to September 10, 1917. *Diet A.* Diabetes gravis. Death. Weight of dog, 3.0 kilos. Weight of pancreas remnant, 1.8 grams.

Dog E. Weight 5 kilos; 21.2 grams pancreas removed June 4, 1917. Pancreas remnant estimated 3.0 grams.

June 15 to 22, 1917. *Meat diet.* No glycosuria.

June 23 to August 1, 1917. *Diet A.* Polyuria (maximum 1000 cc. per day); glycosuria (maximum 40 grams per day).

August 2 to 7, 1917. Starvation. Reduction of polyuria and glycosuria. Urine sugar-free August 6, 7.

August 8 to 16, 1917. *Diet A.* Gastro-intestinal disorders, polyuria, glycosuria and rapid emaciation. Death in coma. Body weight, 3.0 kilos. Weight of pancreas remnant, 2 grams. Skin ulcers and conjunctivitis.

Dog F. Weight 6.8 kilos; 24.3 grams pancreas removed October 27, 1917. Remnant of pancreas estimated 3.7 grams.

October 28 to November 2, 1917. *Diet B.* No polyuria, but glycosuria (maximum 5 grams per day).

November 3 to 16, 1917. *Meat diet.* No glycosuria.

February 17 to 20, 1918. *Diet B.* Glycosuria (maximum 3 grams per day) but no polyuria.

February 21 to June 4, 1918. *Meat diet.* No glycosuria.

June 5 to 9, 1918. *Diet A.* Trace of sugar in urine June 6 and 7, (2.0 grams and 0.3 gram). No glycosuria June 8 and 9. Dog in fine condition. Experiment concluded. Weight of dog, 8.8 kilos. Weight of pancreas remnant, 2.2 grams.

Dog G. Weight 6.7 kilos; 18.3 grams pancreas removed March 7, 1918. Pancreas remnant estimated 3.2 grams.

March 8 to 15, 1918. *Meat diet.* No glycosuria.

March 16 to 19, 1918. *Diet A.* Glycosuria (average 3 grams per day) but no polyuria.

March 20 to June 4, 1918. *Meat diet.* No glycosuria.

June 5 to 8, 1918. *Diet A.* No glycosuria. Dog in fine condition. Experiments concluded. Weight of dog, 8.8 kilos. Weight of pancreas remnant, 6.8 grams.

Dog H. Weight 6.7 kilos; 23.2 grams pancreas removed February 24, 1918. Pancreas remnant estimated 3.2 grams.

February 25 to 28, 1918. *Meat diet.* No glycosuria.

February 29 to March 3, 1918. *Diet A.* Slight polyuria and glycosuria (average 0.7 gram sugar per day).

March 4 to May 23, 1918. *Meat diet.* No glycosuria.

May 25 to 28, 1918. *Diet A.* No glycosuria. Dog in fine condition. Experiment concluded. Weight of dog, 8.9 kilos. Weight of pancreas remnant, 7.7 grams.

Dog I. Weight 7.8 kilos; 22.4 grams pancreas removed October 26, 1917. Pancreas remnant estimated, 3.1 grams.

October 27 to 31, 1917. *Meat diet.* No glycosuria.

November 1 to 4, 1917. *Diet B.* Slight glycosuria (2 grams per day).

November 4 to December 21, 1917. *Meat diet.* No glycosuria.

December 22 to 24, 1917. *Diet B.* Slight glycosuria (1.6 gram per day).

December 25, 1917 to February 5, 1918. *Meat diet.* No glycosuria. Dog died by accident. Body weight, 6.4 kilos. Weight of pancreas remnant, 3.3 grams.

COMMENTS AND CONCLUSIONS

1. In general, our results support the view that a liberal carbohydrate diet tends to change diabetes levis into diabetes gravis, after partial pancreatectomy in dogs. But our experiments do not constitute a clear demonstration of this thesis, in fact they are not much more conclusive than the experiments reported earlier by Thiroloix, and by Allen.

It seems to us that the question can be settled by the statistical method only, using a great many animals for the work, because of the fact that one cannot predict the metabolic course of any one animal deprived of approximately seven-eighths of the pancreas. When a considerably smaller pancreas remnant is left the dog passes into fatal diabetes irrespective of the diet; if the pancreas remnant is somewhat larger no amount of forced carbohydrate feeding induces diabetes gravis. And since the functional efficiency of the pancreas appears to vary in different dogs, the estimation, by weight, of the optimum pancreas remnant for the solution of this question involves a large and variable error.

The analogy from an impaired digestion is not to the point. It is true that ingesting certain foods, or any food in certain amounts, may aggravate the untoward condition of an alimentary tract whose digestive juices and motor power are much below normal, but this is not due to any "overstrain" of the digestive glands, or of the gastro-intestinal

motor mechanisms. It is due to stasis, bacterial fermentation and putrefaction, and to abnormal reflexes evoked from an hyperirritable gut.

2. The view of organ impairment as a direct result of physiological "overstrain" is simple, is readily strengthened by analogy, and, viewed with little or no critical analysis, appears so reasonable that it is being used as sufficient explanation of results in physiology and medicine to a greater extent than the facts warrant. It is true that excessive stimulation of the eye or the ear leads to degeneration in the retinal and cochlear elements. But it is equally true that surgical removal of a large percentage of such glands as the kidney, the thyroid, etc., causes hypertrophy, not atrophy of the gland remnants, unless the gland remnant is so small that conditions are produced in the body which are inimical to growth and repair in general. The facts in regard to the thyroid gland are the most striking in this connection. When one attempts to completely extirpate the thyroid in young animals (rabbits, chickens), it frequently happens that a few thyroid cells (less than $\frac{1}{100}$ part of the gland) are left *in situ*. These few cells invariably multiply up to a point where the gland substance meets the internal needs of the animal. We must assume that when a few thyroid cells are called on to function for the entire thyroid gland, these few cells are being greatly over-stimulated, as compared to the normal. Nevertheless the result is hypertrophy, not atrophy.

3. When the functional capacity of the pancreas remnant is too small to prevent diabetes, the pancreas remnant undergoes practically no hypertrophy. On this point our results are in agreement with those of previous workers. This failure to hypertrophy may be due to the unfavorable influence on growth in general of the diabetic condition, rather than to specific "overstrain" of the pancreas remnant.

4. The favorable effects, at least in many cases, of partial starvation in the management of diabetic patients cannot at present be ascribed, in whole or in part, to relieving an impaired (endocrine) pancreas from the "overstrain" of ordinary diets, in view of the indications from human and comparative physiology that starvation, within limits, tends to rejuvenate tissues in general.

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THE COMPARATIVE PERFORMANCE OF MUSCLES SUBJECTED TO RHYTHMIC AND ARHYTHMIC STIMULATION

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In making records of simple muscle contractions in series to observe the oncoming of fatigue and the final failure of response, it has been customary to send in the stimuli at uniform intervals. It is of interest to find out whether muscles thus treated give a better or a poorer total performance than symmetrical muscles stimulated an equal number of times per minute but with quite irregular spacing of the contractions. If work done is to be our standard, the intervals must in no case be so short as to cause marked summation.

In the present study gastrocnemii of frogs were stimulated directly. The primary current was made and broken as brass brushes trailed on the periphery of brass discs from which segments had been cut. The discs were revolved by a motor, make shocks were excluded by an appropriate device and breaks delivered to the muscle. In one set of discs the divisions of the rim were equal; in the companion set they numbered the same but were of unequal length. In a typical experiment the gastrocnemius from the right leg might be stimulated rhythmically and its fellow from the left leg arhythmically. Graphic records (see fig. 1) were made and the comparative accomplishment measured with a work-adder. The frequency of stimulation varied from 14 to 56 per minute.

A tabulation of fifty experiments, in each of which two symmetrical muscles were matched, shows no significant difference between the results attained in the two types of trial. In solitary cases, differences in the figures for the work done approached 25 per cent but one variety of performance was favored about as often as the other. Generally the muscles compared registered amounts of work which differed by less than 10 per cent. The average of all the experiments gave the following close correspondence. Work done by 50 muscles stimulated at regular intervals, 4255 gram centimeters; by 50 muscles stimulated at irregular intervals, 4276 gram centimeters.

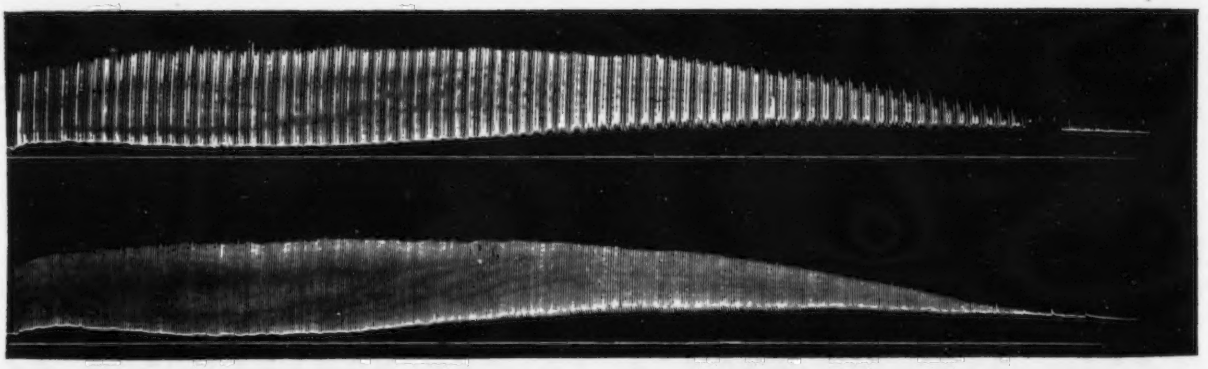

Fig. 1. Experiment 17. The upper tracing shows the contractions of the left gastrocnemius muscle of a frog stimulated arhythmically; the lower tracing, those of the right stimulated rhythmically.

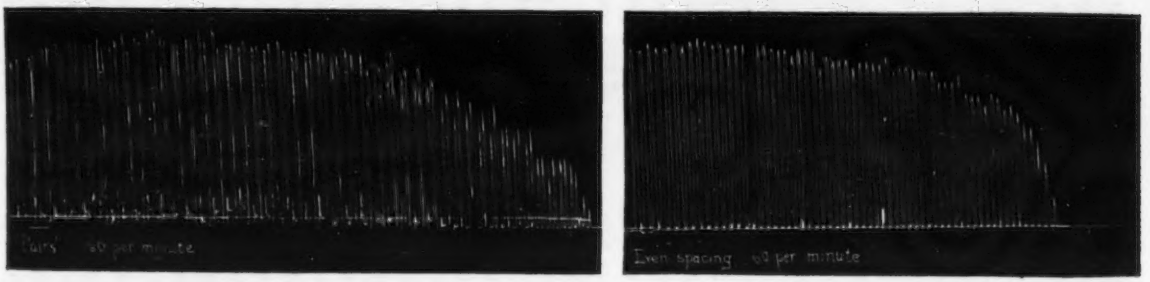

Fig. 2. Illustrating the superiority of an ergograph record with paired contractions. In this case the performance exceeded that with the simple rhythm by about 30 per cent.

The equality noted above naturally raises the question of voluntary contractions. Can one do more work with a weight ergograph when the efforts are made at rigidly uniform intervals or when the intervening periods are alternately long and short? The inquiry has possible bearings upon industrial fatigue. In a series of contractions we may make the interval 2 seconds throughout or it may be alternately 3 seconds and 1 second. Thirty contractions in a minute will be recorded with either procedure. We may group them in other and less simple ways, making three or four efforts in quick succession and thus earning a relatively long rest.

The cue for making the contractions was furnished in our experiments by the appearance from behind a screen of vertical lines borne upon the surface of a revolving drum. These lines presented themselves singly, in pairs, in threes or in fours, as desired, but in every case the number for a revolution of the drum was the same. The frequency was about 50 per minute. The weight, lifted by the middle finger of the right hand with the strap on the distal phalanx, was about 2 kgm. Sometimes a work-adder was connected with the ergograph but the total number of lifts accomplished proved to be a satisfactory criterion.

It soon became obvious that more work can be done when long and short intervals are alternated than when the spacing of the efforts is uniform. The average performance for 12 trials with the contractions in pairs exceeds the average for 16 trials with the contractions evenly spaced by 41 per cent. When the contractions are thrown into groups of three or four with rest periods correspondingly lengthened there is a slight additional gain (fig. 2).

The advantage of such an ordering of work over the simple rhythm appears to be due mainly to a single factor. This is not, as might be anticipated, the longer rest period which keeps recurring; it is, paradoxically, the *shorter* one. The facts seem to be as follows: When a man is compelled to execute a contraction quickly in order to be ready for the next one in its turn *he actually draws less upon his muscular resources than he would if he "took his time."* There is an element in this type of experiment which was not present when the frog muscle was stimulated by single shocks. Voluntary contractions are tetanic in character and of variable duration. The economy of the short, sharp contraction is readily demonstrated. Given 2 seconds in which to contract and relax, one may devote a second to the up-stroke and use the other for the return or it may be possible to get both into the first second and have the remaining half of the time for complete

repose. When the results of these two methods were compared it was found that the first plan led to complete fatigue after 144 contractions while the second did not exhaust the subject in 300 lifts.

It is reasonable to expect a postponement of fatigue with reduced periods of tetanization. If our reasoning is correct one might hope to cultivate a snappy way of executing all contractions which should equalize his rhythmic and arhythmic records. But it is hard to provide an incentive so compelling as the knowledge that a given movement must be finished in time to begin another. In all ergographic studies it must be important to attend to the form of the separate curves as they appear upon a rapidly moving surface. This matter has generally been ignored. There are probably individual peculiarities and it is evident in our own cases that the instinctive mode of raising the weight is not the best attainable from the standpoint of economy. To be hurried to a certain degree proves to be distinctly advantageous.

RENAL ACTIVITY AND THE ACID BASE EQUILIBRIUM

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In order to determine whether changes in the acid base equilibrium of the body influence renal activity, the urea excreting capacity of the kidney was measured after the administration of acid and alkaline sodium phosphate salts. These substances are particularly suitable for this purpose since the same work is imposed on the kidney in the excretion of the phosphate radical, and the only essential difference between them is that the acid phosphate leads to a shift in the neutrality equilibrium toward the acid side, while the alkaline phosphate has the opposite effect. Control experiments can be carried out with a mixture of acid and alkaline phosphate of neutral reaction. The degree of change in the acid base balance is indicated by the extent of the decrease in the carbon dioxide combining power of the plasma after acid phosphate, and the amount of increase which follows the administration of alkaline phosphate.

The ratio between the urea content of the urine and of the blood was taken as the measure of the urea excreting activity of the kidney. The methods and the technique employed by Addis, Barnett and Shevky (1) were followed, involving the collection of four successive samples of urine and of blood over a period of five hours. Five grams of urea were given with the phosphate in order that variations in the ratio arising from other causes than alterations in the neutrality equilibrium should as far as possible be diminished (2). The carbon dioxide combining power of the plasma and the H-ion concentration of the urine were determined during each of the four periods of the test. Van Slyke, Stillman and Cullen's (3) titration method was used for the plasma carbon dioxide, after a parallel series of experiments had shown that in rabbit's plasma essentially the same results were obtained by this method as by the direct evacuation and measurement of the carbon dioxide. Henderson and Palmer's method (4) was used in determining the acidity or alkalinity of the urine, except that 1 cc. instead of 10 cc. of urine was taken.

It was intended that the control and the acid and alkaline experiments should be carried through on a group of twenty rabbits, but five of the twenty died before all the experiments had been completed. On each of the remaining fifteen animals all the experiments described in this and in the succeeding paper were performed and since the comparison of the effects of the various substances given is facilitated by dealing only with this uniform group of fifteen, the partial results obtained on the other five are excluded.

In order that the increase of work thrown on the kidney in the excretion of phosphate should be as far as possible the same, the amounts of neutral, acid and alkaline phosphates given were adjusted so that they contained an equal number of phosphate radicals. It is of course true that this fact does not allow us to assume that there was an equal

TABLE 1

Effect of neutral, acid and alkaline phosphate on the ratio: $\frac{\textit{Urea in one hour's urine}}{\textit{Urea in 100 cc. of blood}}$ *after the administration of urea*

Averages of all periods from experiments on a group of fifteen rabbits

SUBSTANCE GIVEN	PLASMA CO_2	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
				mgm. per hour	*mgm. per 100 cc.*	
Neutral phosphate.....	52	22	6.7	499	161	3.09
Acid phosphate.....	39	15	5.9	299	158	1.86
Alkaline phosphate.....	56	20	6.8	479	157	3.04

absorption of phosphate from the intestine. But Underhill and Bogert (5) were unable to distinguish any difference between the degree of increase in phosphate excretion in the urine produced by the subcutaneous injections into rabbits of acid as opposed to alkaline sodium phosphate. Phosphate estimations in the urine in our experiments would not have been decisive, since we have no means of distinguishing between changes in excretion due to variations in the rate of absorption and those due to variations in the phosphate excreting activity of the kidney. Only the subcutaneous or intravenous injection of the salts would have removed all doubt in regard to this point, but such operative procedures introduce so many variable factors, that the interpretation of the effect on renal function is apt to be obscured.

For the control experiments a mixture of 0.5 gram NaH_2PO_4, $4H_2O$ and 2.32 grams Na_2HPO_4, $2H_2O$ was made. The pH of this mixture

in solution was 7.48. In the acid experiments 3 grams of NaH_2PO_4, $4H_2O$ were given and in the alkaline 2.81 grams of Na_2HPO_4, $2H_2O$. In all cases 5 grams of urea were added to these salts and the whole diluted to 35 cc. with water and administered through a stomach tube just before the bladder was emptied by catheter at the commencement of the collection of the first hour's urine. Although these are large amounts of phosphate no effect on intestinal excretion was observed.

TABLE 2

Effect of neutral, acid and alkaline phosphate on the ratio: $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$ *after the administration of urea*

Averages of separate periods

PERIOD	PLASMA CO_2	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
	1. Neutral phosphate. Control experiments					
	vol. per 100 cc.	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	48	22	7.2	388	144	2.68
2	52	24	6.6	562	171	3.26
3	55	17	6.8	571	168	3.41
4	54	23	6.3	474	160	2.99
	2. Acid phosphate					
1	41	9	6.3	160	116	1.39
2	38	10	5.9	276	156	1.76
3	37	15	5.7	404	181	2.27
4	39	24	5.6	356	177	2.02
	3. Alkaline phosphate					
1	50	17	7.3	341	131	2.60
2	56	21	6.8	517	168	3.07
3	60	18	6.7	580	163	3.55
4	59	22	6.5	480	157	3.07

The average results obtained by combining the four observations made on each of the fifteen rabbits are given in table 1.

A more detailed conception of the effect of acid and alkaline phosphate can be obtained from the average four periods shown in table 2 and charted in figure 1. The data from which these averages were obtained are given in tables 3, 4 and 5.

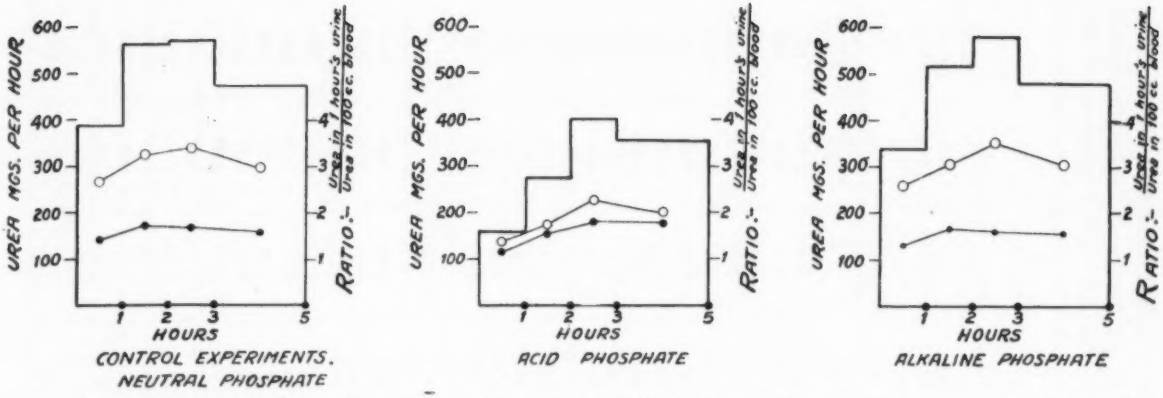

Fig. 1. Showing the decrease in the urea excreting activity of the kidney produced by acid sodium phosphate. The ratio between the urea in one hour's urine and the urea in 100 cc. of blood is shown by circles joined by lines. The value is given on the scale at the right of the charts. The hourly rate of urea excretion is represented by the blocked areas. The blood urea concentration is shown by dots joined by lines. The values for both the rate and the blood concentration are given by the scale on the ordinates at the left of the charts.

These figures show that the shift in the neutrality equilibrium toward the acid side indicated by a reduction in the carbon dioxide combining capacity of the plasma from 52.4 to 38.7 volumes per cent is accompanied by a distinct decrease in the urea excreting activity of the

TABLE 3

Control experiments (neutral phosphate)

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period I			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	50.5	25.0	7.4	340	129	2.63
2	52.3	16.0	7.4	425	165	2.57
3	47.0	14.0	7.5	240	117	2.05
4	50.2	19.0	7.5	400	150	2.67
5	48.0	29.0	7.4	447	126	3.55
9	54.5	20.5	7.6	285	123	2.32
10		22.5	7.0	531	192	2.77
11	46.8	20.0	6.3	266	153	1.74
12	49.3	38.0	7.2	570	165	3.45
13	44.0	21.0	7.0	531	150	3.54
14	54.6	47.0	8.0	301	129	2.33
15	40.4	15.0	6.9	374	114	3.37
16	45.8	26.0	7.4	494	162	3.05
19	45.5	15.0	7.4	375	177	2.12
20	43.5	8.0	6.3	242	111	2.18
			Period II			
1	52.5	24.5	6.3	540	156	3.46
2	51.8	21.0	6.7	598	195	3.07
3	49.4	19.0	6.9	390	150	2.60
4	53.2	18.0	7.4	475	189	2.51
5	55.1	28.0	6.3	544	159	3.42
9	51.8	15.0	7.4	285	150	1.90
10	51.9	24.0	6.3	730	213	3.43
11	53.8	29.0	6.0	564	186	3.03
12	53.4	27.0	6.3	631	180	3.51
13	56.0	23.0	6.3	740	174	4.25
14	57.0	28.0	7.0	460	153	3.01
15	50.3	18.0	6.3	603	165	3.65
16	54.3	38.0	7.4	621	162	3.83
19	50.2	16.0	6.7	620	186	3.33
20	44.8	24.0	6.3	634	168	3.78

TABLE 3—*Concluded*

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period III			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	55.0	17.0	6.3	541	147	3.68
2	50.0	16.0	6.3	659	180	3.66
3	51.6	20.0	6.3	520	150	3.33
4	56.4	18.0	7.0	554	190	2.83
5	60.2	16.0	6.3	494	150	3.29
9	50.5	18.0	7.0	475	168	2.83
10	51.2	15.0	6.3	681	192	3.55
11	55.5	24.0	6.0	546	177	3.09
12	59.1	14.0	6.3	518	159	3.26
13	57.0	20.0	6.3	749	168	4.46
14	57.0	21.0	6.0	530	171	3.10
15	48.0	11.0	6.0	508	153	3.32
16	58.5	15.0	7.0	529	162	3.27
19	54.7	13.0	6.3	599	171	3.50
20	56.5	22.5	5.9	660	168	3.93
			Period IV			
1	55.0	22.0	6.0	413	135	3.06
2	49.2	19.0	6.3	470	177	2.65
3	56.0	26.0	6.3	453	156	2.90
4	60.8	26.0	6.7	505	192	2.63
5	59.0	19.0	6.0	359	153	2.35
9	56.0	26.0	7.0	471	150	3.14
10	51.5	20.0	6.3	509	183	2.78
11	54.6	38.0	6.0	460	171	2.69
12	54.8	20.0	6.3	425	141	3.01
13	56.8	23.0	6.3	585	141	3.98
14	50.2	35.0	6.3	445	174	2.86
15	45.8	16.5	6.0	504	153	3.29
16	54.3	17.5	6.7	469	147	3.19
19	54.7	18.0	6.3	537	165	3.25
20	56.9	21.0	6.0	510	150	3.40

kidney. The urea concentration of the blood is almost the same yet 499 mgm. of urea per hour are excreted after neutral phosphate, and only 299 mgm. per hour after acid phosphate. The average ratio between the urea content of the urine and the blood is thus reduced from 3.08 to 1.86. This is a difference which is much greater than any

which could have arisen by chance. In the four periods the ratios after acid phosphate are less than the ratios in the control experiments by amounts which are respectively 9.9, 11.2, 7.5 and 8.7 times greater than the "probable differences between the averages" (6).

TABLE 4

Experiments with acid phosphate

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period I			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	44.6	8.5	6.0	134	108	1.24
2	36.6	11.0	6.3	296	129	2.29
3	42.6	8.0	6.3	176	102	1.72
4	47.3	8.5	6.0	135	120	1.13
5	42.0	16.0	6.0	263	105	2.50
9	42.5	8.0	6.7	120	135	0.89
10	44.5	6.0	7.4	86	105	0.84
11	33.1	7.0	5.0	96	75	1.28
12	36.6	9.0	6.0	250	138	1.81
13	51.5	12.0	7.0	234	150	1.55
14	40.4	9.0	6.0	134	111	1.21
15	31.7	6.0	5.7	114	93	1.23
16	37.8	8.5	6.3	180	132	1.36
19	41.1	4.5	7.4	71	126	0.56
20	44.6	8.0	6.0	118	105	1.13
			Period II			
1	30.0	8.0	5.7	261	153	1.71
2	37.2	12.0	6.3	405	156	2.60
3	38.1	9.0	6.3	237	132	1.79
4	32.7	8.5	6.0	210	165	1.27
5	41.0	11.0	6.0	299	141	2.12
9	40.3	6.5	6.3	227	177	1.28
10	40.3	13.0	6.3	279	147	1.90
11	32.7	6.0	4.7	120	126	0.95
12	35.8	13.0	5.7	428	171	2.50
13	49.1	14.0	6.0	370	189	1.96
14	38.9	4.0	6.0	177	174	1.02
15	31.3	7.0	6.0	245	135	1.81
16	40.0	20.0	6.0	310	162	1.92
19	37.5	9.0	6.0	204	162	1.26
20	44.6	9.0	5.5	370	156	2.37

TABLE 4—*Concluded*

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period III			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	35.4	15.0	5.7	299	171	1.75
2	37.4	16.5	5.4	564	162	3.48
3	25.3	9.5	6.3	256	144	1.77
4	34.9	15.0	6.0	198	177	1.12
5	42.5	17.0	5.5	425	153	2.78
9	38.6	9.0	6.3	305	237	1.29
10	36.6	15.0	6.2	417	195	2.14
11	34.5	26.0	4.7	593	162	3.66
12	35.8	17.0	5.3	535	189	2.83
13	47.0	18.0	6.0	551	201	2.74
14	42.5	9.0	5.2	358	216	1.66
15	30.8	9.0	5.7	331	150	2.21
16	36.4	22.5	6.0	443	216	2.05
19	36.6	12.5	5.7	282	183	1.54
20	40.2	15.0	5.3	505	165	3.06
			Period IV			
1	37.5	29.0	5.5	205	189	1.09
2	37.9	24.5	5.6	456	159	2.86
3	39.4	30.0	5.7	349	147	2.38
4	37.4	14.0	5.7	187	174	1.07
5	41.5	44.0	5.0	390	165	2.36
9	37.2	16.0	6.0	337	195	1.73
10	44.8	28.0	6.0	318	180	1.77
11	35.8	33.0	4.7	424	183	2.32
12	37.0	16.0	5.7	284	174	1.63
13	46.0	23.0	6.0	480	186	2.58
14	42.0	23.0	5.7	455	213	2.14
15	35.7	21.5	5.8	346	161	2.10
16	38.9	14.0	5.7	396	180	2.20
19	37.5	26.5	5.5	312	180	1.73
20	42.5	22.0	5.3	398	165	2.41

On the other hand it is equally plain from the results shown in tables 1 and 2 that the alkaline phosphate had no appreciable effect on the urea excreting activity of the kidney. The slight and irregular differences between the ratios of the control and alkaline phosphate experiments might well be due to chance. But it will be noted that the car-

bon dioxide content of the plasma is only slightly increased. On account of the lesser solubility of the alkaline phosphate it was impossible to give a quantity which would markedly change the balance toward the alkaline side. All that can be said is that an increase in

TABLE 5

Experiments with alkaline phosphate

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period I			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	51.5	26.0	7.0	445	150	2.97
2	56.5	15.5	8.0	424	150	2.83
3	52.3	14.0	7.7	288	132	2.18
4	58.5	22.5	8.0	339	146	2.35
5	47.0	13.5	7.0	367	126	2.91
9	50.0	16.0	7.7	350	168	2.12
10	55.9	27.0	7.7	520	156	3.33
11	51.8	7.0	5.7	165	60	2.75
12	55.8	20.0	7.4	409	166	2.84
13	44.8	18.0	6.7	400	120	3.33
14	53.6	16.0	7.4	231	111	2.08
15	45.0	18.0	7.0	380	165	2.30
16	48.2	18.0	7.4	180	69	2.61
19	41.5	13.5	7.4	350	147	2.38
20	40.3	11.0	6.7	268	132	2.03
			Period II			
1	59.0	27.5	5.7	566	177	3.20
2	60.9	23.0	8.0	495	165	3.00
3	59.0	22.5	7.0	504	159	3.17
4	58.5	23.0	7.4	410	165	2.49
5	50.5	16.0	6.0	504	165	3.05
9	58.1	18.0	7.0	509	177	2.88
10	55.9	20.5	7.7	455	183	2.49
11	54.5	18.0	6.3	580	177	3.28
12	61.8	25.5	7.0	690	168	4.11
13	60.2	20.0	6.9	670	156	4.29
14	49.2	9.5	7.0	350	141	2.48
15	50.0	18.0	6.3	510	183	2.79
16	53.8	33.0	7.0	393	162	2.42
19	53.6	19.5	6.7	510	177	2.88
20	50.5	20.0	6.3	608	171	3.55

TABLE 5—*Concluded*

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period III			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	63.6	21.0	6.7	631	156	4.05
2	67.0	19.0	7.4	566	159	3.56
3	58.1	16.0	7.0	508	153	3.32
4	58.1	29.0	7.4	555	159	3.49
5	56.4	14.5	5.7	580	156	3.72
9	59.0	15.0	7.0	520	165	3.15
10	56.7	19.0	7.4	580	183	3.17
11	60.2	19.0	6.3	705	183	3.85
12	67.0	17.0	7.0	720	177	4.07
13	66.2	16.0	6.9	710	159	4.46
14	48.4	14.0	6.7	420	150	2.80
15	53.3	15.5	6.3	573	147	3.90
16	60.2	15.0	6.7	598	177	3.38
19	60.5	20.0	6.3	472	168	2.81
20	61.2	14.5	6.3	555	159	3.49
			Period IV			
1	64.3	20.0	6.3	405	150	2.70
2	62.5	23.0	7.0	530	150	3.53
3	58.1	23.0	6.7	455	156	2.92
4	67.2	25.0	7.0	497	171	2.91
5	62.5	18.0	6.0	530	153	3.46
9	55.0	18.0	6.7	454	153	2.97
10	48.3	24.0	7.0	432	174	2.48
11	65.7	28.0	6.3	585	159	3.68
12	65.8	20.0	6.3	436	141	3.09
13	69.2	24.0	6.9	540	141	3.83
14	48.0	33.5	6.3	455	150	3.03
15	46.0	17.5	6.3	440	162	2.72
16	64.6	24.0	6.3	570	183	3.12
19	57.0	19.0	6.3	389	153	2.54
20	56.6	19.0	6.0	475	153	3.11

carbon dioxide combining power of from 52.4 to 56.3 volumes per cent produced by the administration of alkaline phosphate has no definite influence on renal activity.

By giving sodium bicarbonate it is possible to produce a change toward the alkaline side as marked as the change toward acidity,

induced by acid phosphate. The conditions of the experiment are not so uniform since the kidney is under different conditions in excreting an excess of neutral phosphate and an excess of sodium bicarbonate, but we believe the experiment is of value in judging the probable general effect of a pronounced increase in the potential alkalinity of the plasma. The same group of fifteen rabbits was given 3 grams $NaHCO_3$ dissolved with 5 grams of urea in 35 cc. of water. The average results

TABLE 6

Effect of sodium bicarbonate on the ratio: $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$ *after the administration of urea*

Averages of all periods from experiments on a group of fifteen rabbits

PLASMA CO_2	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
vol. per 100 cc.	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
73	26	8.4	461	159	2.91

TABLE 7

Effect of sodium bicarbonate on the ratio: $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$ *after the administration of urea*

Averages of separate periods

PERIOD	PLASMA CO_2	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
	vol. per 100 cc.	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	67	28	8.0	390	144	2.73
2	73	28	8.4	482	164	2.96
3	74	23	8.6	525	167	3.12
4	76	26	8.6	448	161	2.82

are compared with the neutral phosphate controls in tables 6 and 7 and charted in figure 2. The details are given in table 8.

Under the conditions of this experiment a marked shift toward alkalinity is associated with a slight decrease in the urea excreting activity of the kidney. With almost the same urea concentration of the blood 499 mgm. of urea per hour are excreted after neutral phosphate and 461 mgm. per hour after sodium bicarbonate, a ratio of 3.1 is reduced to 2.9. In the first period the ratios are practically identical, but in the succeeding periods when the greatest effect on the carbon dioxide

combining power of the plasma is produced the ratios after bicarbonate are less than the corresponding control ratios by amounts which are respectively 2.0, 2.5 and 1.7 times larger than the probable differences. Although the decrease in none of the periods separately is enough to exclude chance, yet taken together they make it improbable that the divergence is not due to a specific difference in the effects of the neutral phosphate and the bicarbonate. It cannot with certainty be said that this difference is due to the fact that the one is neutral and that the other is alkaline, but the demonstration that acid phosphate depresses renal function on account of its acidity lends support to that interpretation.

I am indebted to Dr. T. Addis and Mr. A. E. Shevky for suggesting work on this subject.

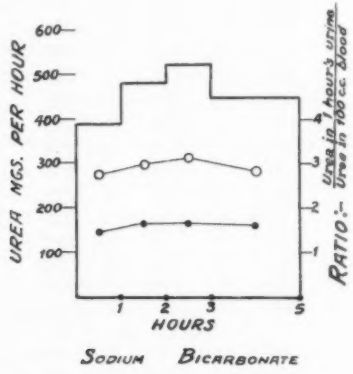

Fig. 2. Showing the effect of sodium bicarbonate.

CONCLUSIONS

1. The urea excreting activity of the kidney under strain was measured after the administration of a mixture of acid and alkaline phosphate of neutral reaction, and also after the administration of an amount of acid phosphate containing the same amount of phosphorus. A distinct decrease in function was observed after acid phosphate. Since the only essential difference in the conditions of these experiments lay in the fact that after the neutral phosphate mixture the acid base equilibrium remained unchanged, whereas after acid phosphate there was a shift toward the acid side, it is concluded that the decrease in the alkalinity of the plasma induced by an increase in the amount of

acid phosphate within the body, decreases the urea excreting activity of the kidney.

2. The administration of an amount of alkaline phosphate containing an amount of phosphorus equivalent to that given in the experi-

TABLE 8

Experiments with sodium bicarbonate

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period I			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	69.7	27.0	7.4	430	168	2.56
2	64.7	30.0	8.7	347	126	2.76
3	77.3	42.0	8.7	350	144	2.43
4	69.4	32.0	8.0	483	126	3.84
5	64.9	46.0	8.0	560	135	4.15
9	78.0	25.0	8.7	359	177	2.03
10	74.0	16.0	8.7	350	183	1.91
11	47.0	7.5	7.2	104	72	1.44
12	58.0	19.0	8.4	365	126	2.90
13	85.0	16.5	8.0	467	129	3.62
14	60.2	53.0	7.6	518	186	2.78
15	64.8	41.0	8.0	492	171	2.88
16	60.2	30.0	8.0	394	144	2.74
19	64.0	14.0	7.4	293	144	2.04
20	71.0	16.0	7.2	345	123	2.80
			Period II			
1	72.0	23.0	8.0	494	177	2.79
2	85.1	44.0	8.7	473	147	3.22
3	72.9	28.0	8.7	489	171	2.86
4	62.7	18.0	8.7	267	141	1.89
5	69.1	33.0	8.0	655	153	4.28
9	84.0	23.0	8.7	465	198	2.35
10	87.1	20.0	8.7	473	192	2.46
11	58.2	12.0	8.5	396	123	3.22
12	78.2	36.0	8.4	537	180	2.98
13	69.0	36.0	8.4	465	132	3.52
14	70.8	28.0	8.7	465	186	2.50
15	78.1	33.0	8.4	629	186	3.38
16	71.5	49.5	8.4	455	162	2.81
19	68.0	16.5	8.4	450	159	2.83
20	73.8	20.5	7.4	513	156	3.29

TABLE 8—*Concluded*

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period III			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	70.0	21.0	8.7	600	189	3.18
2	64.7	30.0	8.7	347	126	2.76
3	72.9	28.0	8.7	489	171	2.86
4	69.4	15.0	8.7	399	141	2.83
5	78.2	16.0	8.7	589	156	2.79
9	80.5	22.0	8.7	558	180	3.10
10	86.0	15.0	8.7	467	204	2.29
11	62.0	18.0	8.7	416	114	3.65
12	79.0	20.0	8.4	585	171	3.42
13	75.0	27.0	8.4	543	150	3.62
14	73.0	21.0	8.7	533	207	2.58
15	74.5	15.5	8.7	505	174	2.90
16	79.0	27.0	8.7	646	189	3.42
19	67.1	16.5	8.7	526	168	3.13
20	82.5	53.5	8.0	674	159	4.24
			Period IV			
1	71.5	29.5	8.0	498	168	2.96
2	79.3	21.0	8.7	353	141	2.51
3	75.0	30.0	8.7	435	156	2.79
4	73.9	23.0	8.7	342	129	2.65
5	72.1	15.0	8.2	439	144	3.05
9	74.0	22.5	8.7	419	183	2.29
10	79.0	23.0	8.7	486	198	2.46
11	62.0	18.0	8.7	416	114	3.65
12	80.5	27.5	8.7	501	153	3.27
13	72.8	30.0	8.7	530	160	3.31
14	82.1	23.0	8.7	404	195	2.07
15	78.5	19.0	8.7	420	162	2.59
16	80.5	32.0	8.7	485	180	2.70
19	71.2	18.0	8.7	417	168	2.48
20	80.5	54.0	8.4	580	168	3.45

ments with neutral and acid phosphates, only slightly increased the alkalinity of the plasma and had no appreciable effect on renal function.

3. The administration of amounts of sodium bicarbonate which markedly increased the alkalinity of the plasma was accompanied by a slight decrease in the urea excreting activity of the kidney.

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THE UREA EXCRETING ACTIVITY OF THE KIDNEY AND PHOSPHATE EXCRETION

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The quantity of 5 grams of urea given in the experiments on rabbits detailed in the preceding paper increases the blood urea concentration to an abnormally high level. The kidney responds with a rate of work in urea excretion considerably higher than any required under physiological conditions. And when, as in the above experiments, there is at the same time a pronounced increase in the amount of phosphates, another strain, also exceeding in extent any previously experienced, is suddenly thrown on the excretory capacity of the kidneys. Under these conditions it is of interest to determine whether the urea excreting capacity is altered on account of the simultaneous increase in phosphate elimination.

It has been shown (1) that a moderate increase in the urea content of the blood is followed by a proportionally greater increase in the urea content of the urine. Evidence has been given that this increase in excretory activity is brought about through the intervention of extrarenal factors under the control of the nervous system, which are able to augment the effectiveness of the anatomical mechanism concerned with urea excretion (2). But there is a limit to the action of these compensatory factors, for with a continued increase in blood urea concentration, the ratio between the urea content of the urine and of the blood increases more and more slowly. After a level of 150 mgm. of urea per 100 cc. of blood is reached, there is very little change in the ratio, and there is a definite decrease at concentrations above 225 mgm. per 100 cc. At the point at which the ratio ceases to increase, the rate of urea excretion has attained its maximum; any call for further work in the form of a still greater increase in the urea content of the blood is unavailing and leads only to a decrease in the ratio. Under these conditions the kidney becomes insufficient because the structural mechanism underlying urea excretion has already attained its fullest capacity for work (3).

In this paper the ratio after 5 grams of urea is compared with the ratio after 5 grams of urea given with a neutral mixture of acid and alkaline sodium phosphate. In both experiments the blood urea concentration rose after the first period to levels between 160 and 175

TABLE 1

Comparison of the effect of urea alone and of urea given with neutral phosphate on the ratio: $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$

Averages of all periods from experiments on fifteen rabbits

SUBSTANCE GIVEN	PLASMA CO_2	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
	vol. per 100 cc.	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
Urea	51	20	6.3	426	164	2.61
Urea + neutral phosphate	52	22	6.7	499	161	3.09

TABLE 2

Comparison of the effect of urea alone and of urea given with neutral phosphate on the ratio: $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. of blood}}$

Averages of separate periods

PERIOD	PLASMA CO_2	VOLUME OF URINE	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
1. Urea						
	vol. per 100 cc.	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	48	16	7.0	283	141	2.00
2	53	19	6.2	443	171	2.64
3	52	18	6.0	512	175	2.98
4	52	26	5.8	466	167	2.82
2. Urea + neutral phosphate						
1	48	22	7.2	388	144	2.68
2	52	24	6.6	562	171	3.26
3	55	17	6.8	571	168	3.41
4	54	23	6.3	474	160	2.99

grams of urea per 100 cc. If with such amounts of urea in the blood the ratio is lower when phosphate is given with the urea than when urea is given alone, that fact would be in favor of the hypothesis of a common anatomical mechanism for the excretion of both urea and phosphate, a mechanism which was unable to excrete the urea so

rapidly when it was simultaneously required to deal with the elimination of a large amount of phosphate. If on the other hand the ratio is found to be unchanged when phosphate is given with the urea, such a result might be taken as evidence of the existence of separate mechanisms for urea and phosphate excretion. And finally, an increase in the ratio obtained when phosphate is given with the urea would tend to show in addition that extra-renal factors augmenting excretory activity are more fully mobilized under the double need for elimination.

The same group of 15 rabbits on which the ratio had been measured after 5 grams of urea given with neutral phosphate, was subjected to another series of experiments in which 5 grams of urea alone were administrated. The conditions otherwise were the same. The aver-

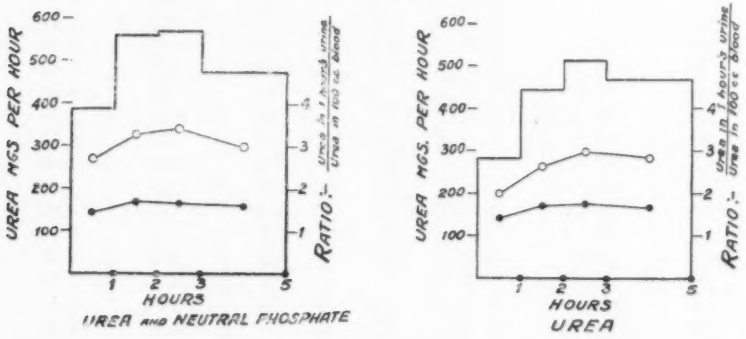

Fig. 1. Showing a slight increase in the urea excreting activity of the kidney when neutral phosphate is given with urea.

ages for the combined and separate periods are shown in tables 1 and 2 and the individual experiments are detailed in table 3 at the end of the paper.

The ratios after urea and phosphate for the four successive periods exceed the ratios after urea alone by 0.68 ± 0.15, 0.61 ± 0.15, 0.42 ± 0.12 and 0.11 ± 0.17 respectively, i.e., by amounts which cannot well be explained by chance in view of the fact that in all four periods the ratios are greater when both substances were given. The plasma carbon dioxide in both cases was almost the same, so that the effect cannot be accounted for on the basis of a change in the acid base equilibrium. Additional experiments were carried out under the same conditions in order to determine the degree to which phosphate excretion was increased after introducing a balanced mixture of acid and alkaline

phosphate into the stomach. An average hourly excretion of 2.6 mgm. P. was found during the five-hour period when urea alone was given, and 8.0 mgm. when phosphate was given with the urea. The urea excreting activity of the kidney was thus increased while at the same time additional work was being done in the excretion of phosphate.

TABLE 3

Urea alone

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period I			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	51.9	18.5	6.3	340	150	2.18
2	39.2	10.0	5.4	290	177	1.64
3	47.5	13.0	6.6	233	123	1.89
4	54.2	20.0	8.0	255	156	1.64
5	49.1	28.5	7.0	495	135	3.67
9	49.3	18.0	7.4	323	204	1.58
10	50.1	17.0	7.4	204	153	1.34
11	45.0	8.0	5.5	132	99	1.34
12	40.2	9.0	7.4	120	78	1.54
13	70.2	25.0	8.7	408	132	3.09
14	48.2	7.5	7.4	228	123	1.86
15	40.3	14.0	7.0	350	150	2.33
16	44.6	21.0	7.5	220	114	1.93
19	40.2	15.0	7.0	360	160	2.14
20	51.5	17.0	7.0	292	153	1.91
			Period II			
1	51.2	18.5	5.0	350	165	2.12
2	50.4	15.0	6.7	474	189	2.51
3	50.4	13.0	6.0	339	171	1.98
4	56.0	23.0	7.7	500	177	2.82
5	52.3	32.0	4.7	640	150	4.27
9	52.4	19.0	7.0	525	216	2.43
10	49.3	11.5	6.7	233	216	1.08
11	47.2	13.0	4.7	426	150	2.84
12	51.5	27.5	4.7	368	146	2.55
13	71.5	13.0	8.0	450	150	3.00
14	52.1	8.5	6.0	411	150	2.74
15	55.7	16.0	5.6	570	177	3.22
16	56.0	44.0	8.0	414	177	2.34
19	51.0	14.0	5.3	466	171	2.73
20	52.8	18.0	6.3	485	168	2.89

TABLE 3—*Continued*

RABBIT	PLASMA CO_2	URINE VOLUME	pH OF URINE	UREA IN URINE	UREA IN BLOOD	RATIO
			Period III			
	vol. per cent	*cc.*		*mgm. per hour*	*mgm. per 100 cc.*	
1	51.2	20.0	5.0	502	177	2.84
2	47.0	14.0	5.8	519	189	2.75
3	47.3	12.0	6.0	377	177	2.13
4	58.9	19.0	7.0	606	186	3.26
5	54.9	21.5	4.7	621	147	4.22
9	51.3	17.0	7.2	539	219	2.46
10	48.0	15.0	6.3	402	222	1.81
11	47.0	15.5	4.7	510	162	3.15
12	50.3	30.5	4.7	481	153	3.15
13	64.5	11.5	7.6	486	150	3.24
14	52.1	11.0	6.0	521	150	3.47
15	57.2	14.0	6.0	595	180	3.30
16	54.0	30.0	8.0	573	180	3.18
19	45.0	17.0	5.0	452	168	2.69
20	53.5	19.0	6.0	490	159	3.08
			Period IV			
1	50.0	41.0	5.0	430	171	2.51
2	53.8	16.0	4.7	440	186	2.36
3	47.5	16.5	6.0	365	165	2.21
4	62.7	20.0	6.7	426	180	2.37
5	53.3	22.0	6.7	527	147	3.59
9	54.9	30.0	6.7	558	192	2.87
10	48.7	36.0	6.0	520	213	2.44
11	50.4	22.0	6.7	495	146	3.44
12	50.1	28.0	6.7	393	162	2.43
13	64.5	19.0	7.0	420	150	2.80
14	51.2	20.0	6.0	560	150	3.73
15	47.8	21.0	6.0	506	153	3.31
16	48.1	40.0	7.7	563	189	3.98
19	49.2	27.0	6.7	345	168	2.05
20	49.3	24.0	6.0	456	141	3.24

CONCLUSION

The urea excreting activity of the kidney measured during the strain induced by the administration of urea is increased when an additional strain is put on the excretory capacity by the simultaneous administration of a neutral mixture of acid and alkaline phosphate.

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GASTRIN STUDIES

III. The Response of the Stomach Mucosa of Various Animals to Gastrin Bodies

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In the two previous reports (1), (2) published on the distribution of gastrin, we called attention to the characters of the responses of various types of stomachs and determined the distribution of this body (or bodies) in the various tissues.

This method of stimulation of the gastric mucosa gives us an opportunity to investigate some of the more fundamental problems of gastric physiology which can not be attacked through food stimulation. Consequently it is important to extend observations on its secretogogue activity to the more common laboratory animals. If the response proves to be a general property of stomach tissue then generalizations based upon it have an added significance.

I. GASTRIN STIMULATION

Cats. In a former paper (1) two observations on cats with Pawlow stomachs showed no increase in the rate of secretion of gastric juice after gastrin injection, but a distinct increase in the pepsin concentration. In the table below (table 1) it is definitely shown that cats respond not only as to increase in concentration of acid but also as to volume of gastric juice.

It will be noted that cat I failed to respond until the cord had been sectioned; cat II also presented two negative experiments. Since these studies were undertaken earlier in the work using less active preparations, they are to be considered rather as evidence that the stomach of the normal cat is much more refractory than that of the dog. The insusceptibility of the cat's stomach comes into evidence

again, when they are dressed, and one collects the resulting spontaneous secretion. For it is the rule that the juice obtained consists of viscid neutral mucus, varying in quantity from 1 cc. per hour to 1 cc. collected in 4 to 5 hours. The absence of acid in this spontaneous secretion is in striking contrast to the conditions found in dogs; for in a dog with a gastric fistula one may at times by simply dressing observe the presence of a secretion of 20 to 30 cc. of juice per hour containing

TABLE 1

Gastric fistulae

	CAT I. CORD CUT AT 2ND THORACIC			CAT III. DOUBLY VAGOTOMIZED			CAT IV. NORMAL			CAT V. NORMAL		
	Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid	
		Free	Total		Free	Total		Free	Total		Free	Total
	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent	cc.	per cent	per cent
Hour preceding	2.5	0.31	0.35	1.6	0.02	0.10	1.9	0.00	0.03	1.0	0.10	0.17
Dose	1 cc. of gastrin (SI)			1 cc. of gastrin (Sh,p. 40)			1 cc. of gastrin (Sh,p. 40)			1 cc. of gastrin (Sh,p. 40)		
First hour	10.0	0.47	0.51	9.8	0.39	0.43	9.5	0.28	0.32	9.8	0.37	0.43
Second hour	10.0	0.53	0.54	3.5	0.33	0.41	2.2	0.30	0.37	7.8	0.47	0.53
Third hour	4.6	0.50	0.53	1.0	0.20	0.27	1.8	0.18	0.26	3.0	0.44	0.47

Additional data:

Cat I. Failed to respond to 1 injection of gastrin preceding the section of cord. Three other experiments after cord was sectioned confirm the one cited above.

Cat II. Two negative experiments.

Cat III. Two other confirmatory experiments.

Cats IV and V. One other confirmatory experiment on each. The gastrin solutions used here have been freed to a great extent from proteins by alcohol and basic lead acetate precipitations.

0.5 per cent hydrochloric acid, and it may require 2 to 3 hours for this secretion to subside.

This refractory condition following injections may be simply due to vascular changes, as is suggested later in the discussion.

Attention is called to cat III which was doubly vagotomized and which confirms the activity of this type of stomach in dogs as previously reported.

Rabbits. Most observers are familiar with the great difficulty experienced in freeing the stomach of these animals of food. It was our practice to sew the stomach of the animal to the abdominal wall over a fairly wide area and then on the following day to make an opening into the lumen by means of a cautery. If the opening is sufficiently large the stomach can be eventually emptied by the use of a fine stream of water. These animals responded readily in our experiments, two of which are cited.

Rabbit I. July 10

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	*per cent*	*per cent*
3:30	Stomach washed out		
4:10	3.0	0.00	0.02
4:10	Injected 1 cc. gastrin "Sh filtrate, p. 40" intramuscularly		
5:10	26.4	0.17	0.20
6:10	25.0	0.36	0.41

Rabbit II. July 12

3:00	Stomach washed out		
4:25	Collection begun		
4:55	7.5	0.00	0.04
5:25	7.7	0.00	0.03
5:37	Injected 1 cc. gastrin "Sh filtrate, p. 40" intramuscularly		
6:37	33.0	0.20	0.27
7:37	25.0	0.12	0.19

Guinea pigs. Much difficulty was experienced in obtaining even qualitatively satisfactory experiments on the guinea pig. The emptying of the stomach is attended with a good deal of shock, so that the animals remain rather quiet and depressed after the manipulation. Furthermore, they also more readily show toxic manifestations from the injections. This may possibly be explained as due to improper gradation of the dose.

The following experiment is offered as one of qualitative value in establishing the activity of the preparation.

In view of the difficulties mentioned above it was not considered necessary for our present purposes to seek for more conclusive results.

In the control hour preceding the injection the quantity of juice was 2.5 cc. with an acidity too small to titrate; in the hour following 4.5 cc.

were secreted with a total acid reaching 0.11 per cent. A second injection of double the amount was then given with no increase in quantity, but a further rise in total acid to 0.16 per cent.

Ducks. Ducks were selected for the avian stomach because of their large size and the relative ease with which they can be kept in captivity. A fistula was made into the crop and a rubber tube inserted which tube could be removed at the time of the experiment. Food was removed from the cages the night preceding the experiment. At stated intervals (half-hour or hour periods) the proventriculus was aspirated with a soft perforated gum rubber tube attached to a syringe. The protocols below show definite time intervals but these are to be considered approximations, for often 10 to 12 minutes were consumed in manipulations attendant upon the aspirations. One duck (no. II) died the night following the injection of a rather heavy dose, the others remained in excellent condition. The breast muscles offer a convenient site for the injection.

Duck I

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	*per cent*	*per cent*
9:15	Stomach aspirated; nothing returned		
9:15	2 cc. gastrin "SO" intramuscularly		
10:15	8.5	0.12	0.20
11:15	10.2	0.22	0.26
12:15	4.9	0.20	0.24
	Duck II		
8:30	Stomach washed out		
9:30	1.0	0.06	0.12
9:30	3 cc. gastrin "SO" intramuscularly		
10:30	27.0	0.20	0.26
11:30	26.0	0.24	0.28
12:30	12.5	0.25	0.31
	Duck III		
8:35	Stomach washed out		
9:35	0.5	0.00	0.09
9:35	2 cc. gastrin "SO" intramuscularly		
10:05	0.7	0.37	0.45
10:05	1 cc. gastrin "SO" intramuscularly		
10:35	15.6	0.32	0.37
11:35	28.0	0.21	0.26
12:35	25.0	0.18	0.23

These active responses as shown in the protocols present all the characteristics as to quantity, acid and duration, of the mammalian secretion.

Turtles. With these animals our results have been entirely negative. First intravenous injections into the abdominal veins of pithed preparations were made and the stomach tested at varying intervals with congo red paper. Next several animals were injected intramuscularly with a known active preparation, and after varying intervals pithed. The stomach contents were then tested with congo red paper. In no case was an acid reaction developed. We then in routine autopsied all turtles used in the course of a rather extended piece of pharmacological research in progress in the same building and tested their stomachs for acid. These animals were fed liver daily and their stomachs always contained pieces thereof, but we were never able to demonstrate an acid reaction in the liver debris or in the mucus on the stomach wall. In the light of these findings we feel that our negative results are to be attributed to the resting state of the stomach in captivity and to the hot weather during which we performed the experiments.

Frogs. Gastric fistulae were made in large bull frogs, measuring approximately 30 to 36 cm. in length. These keep well in captivity, the wound healing readily. Indeed if they are not used regularly the fistula will close entirely. Injections were made into the dorsal lymph sac.

October 23, 1916

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	*per cent*	*per cent*
12:25	0.3 glairy mucus, acid to congo red, aspirated with a perforated rubber on a syringe		
12:25	1.25 minims gastrin "SO" in dorsal lymph sac		
1:25	1.9	0.26	0.32
2:25	1.7	0.29	0.35
3:25	0.4	0.16	0.25
	October 24, 1916		
10:30	Nothing returned by aspiration. Stomach then wiped out with a pledget of congo red paper, no reaction		
10:35	1.66 minims gastrin "SO" in dorsal lymph sac		
11:35	2.4	0.22	0.29
12:35	1.4	0.27	0.30
3:30	1.3	0.23	0.30

The above experiments present conclusive evidence of the secretory response of the frog's stomach.

A number of smaller frogs such as are customarily used in laboratory work were injected, afterwards pithed and stomach tested with congo red paper, but in no case was a positive response noted.

Goat.[1] A female goat was operated on November 16, 1915, with the establishment of an Heidenhain stomach. A total of five experiments was performed on the animal in the course of the next month.

TABLE 2

Histamine-di-hydrochloride injected intramuscularly

	PAWLOW ACCESSORY STOMACHS											
	Dog I			Dog II*			Dog X			Dog VIII		
	Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid	
		Free	Total		Free	Total		Free	Total		Free	Total
	cc.	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*
Hour preceding	0.6	0.04	0.07	0.8	0.05	0.12	6.0	0.29	0.31	2.0		0.3
Injection	4 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride		
First hour	3.8	0.26	0.31	4.5	0.26	0.36	8.5	0.47	0.50	9.0	0.38	0.48
Second hour	4.0	0.50	0.55	3.5	0.39	0.45	2.5	0.12	0.13			
Third hour	0.5	0.40	0.46	0.2								

* Heidenhain stomach.

The response was in no case striking. However, there was constantly a slight increase in the acid and an inconstant increase in pepsin and quantity output following the injection. The evidence seems sufficient to warrant the statement that some stimulation really occurred.

[1] The authors are deeply indebted to A. F. Schalk of the Veterinary School of the North Dakota Agriculture College for these experiments and the preparation of the test animal.

II. HISTAMINE STIMULATION

The character of the response under histamine resembles closely that given by the gastrin preparations as shown by tables below. While as yet no attempt has been made to make a comparative study of the phenomena associated with the injections of the two bodies, since such a study would be more valuable when a pure gastrin product is obtained; still it has often seemed that the subjective symptoms associated with histamine administration were more pronounced. The

TABLE 3

Histamine-di-hydrochloride injected intramuscularly

	DOG XI. GASTRIC FISTULA			DOG IV. GASTRIC FISTULA VAGI CRUSHED ONE YEAR PREVIOUSLY			DOG V. GASTRIC FISTULA VAGI CRUSHED ONE YEAR PREVIOUSLY			DOG VI. VAGOTOMIZED GASTRIC FISTULA			DOG VII. VAGOTOMIZED GASTRIC FISTULA		
	Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid		Juice	Hydrochloric acid	
		Free	Total		Free	Total		Free	Total		Free	Total		Free	Total
	cc.	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*
Hour preceding..	12.6	Neg.	+	8.5	0.36	0.42	9.5	0.37	0.43	7.0	0.11	0.25	3.6	0.11	0.18
Dose.....	1 mgm. histamine $(HCl)_2$			1 mgm. histamine $(HCl)_2$			1 mgm. histamine $(HCl)_2$			0.5 mgm. histamine $(HCl)_2$			0.5 mgm. histamine $(HCl)_2$		
First hour...	54.5	0.50	0.54	62.5	0.43	0.47	25.5	0.42	0.47	16.8	0.25	0.34	18.5	0.26	0.33
Second hour...	14.0	0.45	0.48	35.0	0.45	0.50	2.0			5.6	0.26	0.39	1.7	0.28	0.31

animals were invariably restless and showed noticeable evidences of discomfort for 15 to 30 minutes following the injection. Superficial vasodilatation as shown by the reddened nose, conjunctiva and mucosa of the mouth always was definite and frequently marked.

The limits of the dosage have not been determined accurately, but it is safe to say that 0.5 mgm. histamine-di-hydrochloride will produce a satisfactory response as shown by dogs VI and VII. These two animals had been doubly vagotomized, a procedure which our experience has taught us always renders the dog's stomach more refractory.

Cats. Two positive experiments are recorded below.

Cat III (July 19, 1916) doubly vagotomized

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	per cent	per cent
1:25	Dressed		
2:25	1.0	0.19	0.35
3:05	1 mgm. histamine-di-hydrochloride intramuscularly		
4:05	13.0	0.22	0.29
5:05	6.2	0.45	0.48

Cat V. Normal (August 7, 1916)

3:00	Dressed		
4:00	2.2		0.03
4:00	1 mgm. histamine-di-hydrochloride intramuscularly		
5:00	12.0	0.21	0.26
6:00	9.0	0.39	0.45

Rabbits. Rabbits appear to present a slight peculiarity in the time element of the secretion. By references to table 4 it will be noted that the maximum quantity as well as the high point in the acid comes in the second hour. We have no suggestion based on our observations to offer in explanation. However the question is discussed more completely later. Negative results were obtained in case of one animal (no. II) which was in such poor condition that he died a few hours after the injection. So we feel that this particular case is without significance.

Guinea pigs. The experimental difficulties in handling these animals have already been discussed. Two experiments of qualitative value are presented.

In the first case a control period of 3 hours showed a rate of 0.2 cc. with no titratable acid, the hour following an injection of 1 mgm. histamine hydrochloride gave 0.4 cc. juice with 0.11 per cent free and 0.15 per cent total hydrochloric acid.

In a second experiment the control period showed no acid while the hour following the injection gave 0.07 per cent free and 0.11 per cent total hydrochloride acid. There was no increase in quantity.

TABLE 4

Histamine-di-hydrochloride injected intramuscularly

	RABBIT III			RABBIT III			RABBIT IV		
	Quantity	Hydrochloric acid		Quantity	Hydrochloric acid		Quantity	Hydrochloric acid	
		Free	Total		Free	Total		Free	Total
	cc.	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*
Hour preceding	0.2			4.0	0.14	0.21	0.8		
Injection	1 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride			1 mgm. histamine-di-hydrochloride		
First hour	1.8	0.15	0.33	4.6	0.30	0.36	7.8	0.25	0.37
Second hour	11.0	0.36	0.48	30.0	0.39	0.45	14.5	0.43	0.51
Third hour	12.0	0.32	0.47						

Ducks. The stimulation of the avian stomach is shown in the following protocols.

Duck I. October 1, 1916

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	cc.	*per cent*	*per cent*
7:45	Stomach aspirated, nothing returned		
8:00	0.5 mgm. histamine-di-hydrochloride		
8:30	4.2	0.42	0.29
8:30	0.5 mgm. histamine-di-hydrochloride		
9:30	22.0	0.24	0.29
10:30	4.5	0.16	0.23

Duck III. October 1, 1916

8:05	Stomach aspirated, nothing returned		
8:10	1 mgm. histamine-di-hydrochloride		
9:10	10.0	0.21	0.26
10:10	3.8	0.06	0.14

Frogs. The experiments were conducted as previously described and with the following results.

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	October 25, 1916		
	cc.	*per cent*	*per cent*
9:40	Stomach aspirated, nothing returned. Wiped out the stomach with a pledget of congo red paper and no acid reaction shown		
9:50	$\frac{1}{15}$ mgm. histamine-di-hydrochloride		
10:50	0.5	0.20	0.26
12:00	0.9	0.23	0.29
12:00	$\frac{3}{15}$ mgm. histamine-di-hydrochloride		
1:00	0.9	0.27	0.30
2:00	1.0	0.23	0.29
	October 26, 1916		
9:30	Stomach aspirated, nothing returned. Wiped out the stomach with a pledget of congo red paper, no acid reaction		
9:30	$\frac{3}{15}$ mgm. histamine-di-hydrochloride		
10:30	1.0	0.20	0.24
11:30	1.4	0.27	0.30
1:45	0.3		

III. TYRAMINE HYDROCHLORIDE

Although the experiments using this compound are incomplete and inconclusive, yet it seemed best to report our findings to date. It is our intention to complete them as soon as a sufficient quantity of the compound can be synthesized.

Protocols of two positive experiments follow:

TIME	JUICE	HYDROCHLORIC ACID	
		Free	Total
	Cat III. August 18, 1916. Doubly vagotomized		
	cc.	*per cent*	*per cent*
1:10	Dressed		
2:10	0.7	0.04	0.13
2:25	5 mgm. tyramine hydrochloride (Hoffmann-La Roche)		
3:25	6.5	0.19	0.27
4:25	1.2	0.17	0.24
5:55	2.6	0.10	0.18
	Cat IV. August 9, 1916. Normal		
10:30	Dressed		
11:30	0.4	0.00	0.01
12:00	5 mgm. tyramine hydrochloride (Hoffmann-La Roche)		
1:00	5.0	0.13	0.16
2:00	2.5		0.06
3:00	0.5		

Other experiments with tyramine hydrochloride on cats may be summarized thus:

Cat 3. Dose 7½ mgm. positive; 2½ mgm. negative.
Cat 4. Dose 7½ mgm. positive; 2½ mgm. negative.
Cat 5. Dose 5 mgm. negative.
Cat 6. Dose 7½ mgm. positive; 5 mgm. questionable; 2½ mgm. positive.

DISCUSSION

In our two former papers (1), (2) on the distribution of gastrin activity, we reported that it occurs in approximately the same concentrations in stomachs and duodenal mucosa and in the thyroids, providing we do not try to remove the depressor activity by absolute alcohol extraction. Using the same methods we concluded that at times, and for as yet unknown reasons, we find even higher concentrations of the gastrin activity in the liver and pancreas.

We here find the gastric secretory mechanisms of various animals to be stimulated to more or less extent by both histamine and gastrin solutions when injected intramuscularly. In the turtle we were not able to bring about secretion by either substance or by food.

The guinea pig reacted very irregularly and poorly. This may be due to the extreme susceptibility of this animal to histamine as shown by other observers. Thus, Dale and Laidlaw (3) call attention to the very rapid death after the intravenous injection, and conclude that death is due to constriction of the bronchioles together with increased pulmonary pressure. This pulmonary involvement could however be avoided and an actual general rise in blood pressure due to general vasoconstriction could be observed if previous to the injection of histamine the animal were kept under prolonged anesthesia. Other observers have noted diametrically opposite physiological results on various animals as regards blood pressure and also distinctly variable results as to contraction and relaxation of smooth muscle preparations from various organs when treated with histamine. Thus Barbour (4) considers histamine to cause a fall in blood pressure particularly in carnivora, and that positive or negative results on isolated smooth muscle preparations by epinephrin, tyramine and histamine are determined by the quantitative character of the vasoconstrictor and vasodilator fibers to the particular organ in question. It is well known that the virgin or non-pregnant uterus of the rabbit, dog, ferret, monkey or man always contracts after adrenalin treatment

whereas in the cat, guinea pig, and rat this may cause a relaxation and that only the pregnant uterus is caused to contract. The same theory as to the predominance of one or the other form of nerve endings has frequently been resorted to in attempts to explain these results. Cow (5), however, postulates that in the pregnant animal the pituitrin concentration in the blood is higher and hence the pregnant uterus reacts more readily to injected adrenalin or pituitrin. In other words a previous "sensitization" of the tissues with pituitrin (histamine?) (13) renders smooth muscle more susceptible to uniform action by pilocarpine, ergot, ergotoxin, pituitrin, tyramine and adrenalin. Cow has actually shown that by previous feeding of pituitary substance or by previous treatment with pituitrin solutions the non-pregnant uterus of the cat can be made to react like a pregnant uterus when later treated with the drugs named. Just how this "sensitization" is brought about, or whatever the action may be, has not been explained as far as we know. Fröhlich and Pick (6) made observations along somewhat similar lines, but with entirely different and less consistent results. Handowsky and Pick (7) conclude from their studies with the Trendelenburg frog muscle method that histamine (1: 1000), tyramine (1: 1000) and Witte's peptone (1: 100) all act as vasodilators on this preparation, providing the muscle is first put into the vasoconstrictor condition. While these observations do not bear directly on the point in question here, still they are suggestive and some such factors as referred to may explain our peculiar results with guinea pigs and rabbits.

In rabbits we observed less toxic action by gastrin than in guinea pigs, but the time of maximum gastric secretion after histamine is delayed until the second hour, whereas in most of the animals studied we find the maximum rate of secretion in the second thirty minutes after the injection. Possibly the delay may be attributed to the earlier vascular changes leading to an anemia around the glands and that the otherwise favorable secretory action could not manifest itself until later. With gastrin, however, the rabbits show their maximum secretion in the first hour or at any rate a response of the same order as that of the second hour. These observations suggest that gastrin solutions do not contain histamine or, if they do, that the physiological action thereof is modified by some other constituent. If our interpretation of the delay is correct we may restate the proposition by saying that the gastrin solutions do not contain a substance which produces vascular changes unfavorable to secretion. Possibly the more refractory state of the cat's stomach is attributable to these same vascular factors, that is, initial vasoconstriction.

The observations by Dale and Richards (8) indicate a remarkable vascular action by histamine. Apparently it acts upon two distinctly different anatomical structures in the vascular system. It causes a transient vasoconstriction in the arterioles and a more delayed and prolonged vasodilating action on the capillaries due to inhibition of their tone. The main action then consists in opening up new capillary areas together with permeation of the capillary wall by blood plasma. This latter property enables us to understand how the optimum vascular conditions for secretion may be produced by histamine. Dale and Richards' observations indicating a loss of plasma and an increase in concentration of red corpuscles in the blood, as a result of histamine, have also been observed by Simon (9), who noted an eosinophilia after injection of pancreatic secretin solution. Downs and Eddy (10) also recently confirmed the findings of Dale and Richards in that they noted a distinct increase in the concentration of the formed elements in the blood after partaking of food and also after the injection of pancreatic secretin. However, Simon, as well as Downs and Eddy, consider their results as due to an actual increased production of blood cells.

We do not claim that histamine has a specific stimulating effect on gastric secretion for it is well established by Dale and Laidlaw (11) that the salivary glands as well as the pancreas are stimulated to secretion by histamine. There are other studies which indicate a very close similarity if not an identity between histamine, pancreatic secretin and gastrin. Thus, Barger and Dale (12) using a 0.1 per cent HCl extraction, report the isolation of "crystals quite similar to histamine dipicrate" from the small intestines of the ox, under conditions which precluded post-mortem bacterial changes in the tissue. Various observers have considered this as showing that histamine is identical with secretin, although the proof is by no means conclusive either for or against this view. Similarly one might argue that gastrin and histamine are identical and particularly so in view of the recent observations of Abel and Kubota (13), who isolated histamine picrate from gastric mucous membrane.

In regard to the question of the identity of pancreatic secretin and histamine we have the findings of Dale and Laidlaw (11). These authors report one experiment in which it is shown that if doses of histamine and pancreatic secretin which depress the blood pressure to the same level be injected, then the flow of pancreatic juice resulting from the injection of secretin is much greater than that resulting from histamine stimulation. They were, however, not able to prepare a secre-

tin solution free from depressor action. We have not studied the relation of blood pressure to gastric secretion as a result of gastrin and histamine since all of our experiments were made without the use of anesthetics. We have, however, the general impression that histamine is more toxic and causes more marked peripheral vascular changes when we compare equivalent doses from the standpoint of secretagogue action. These general observations together with others noted above and to be reported in later papers, lead us to believe that histamine and gastrin probably are not identical.

We have no conclusive data to offer as to the action of tyramine. Its more definite vasoconstrictor action probably outweighs any secretory stimulating power that it possesses. Our two positive experiments merely suggest that if the vascular conditions were otherwise right for secretion, the response probably would always be positive.

CONCLUSIONS

1. Gastrin and histamine cause gastric secretion in the dog, cat, rabbit, guinea pig, duck and frog.

2. Gastrin causes secretion in goat; histamine has not been tested on this animal.

3. The turtle's stomach did not contain at any time free acid (as tested by congo red) following injection of gastrin intravenously and intramuscularly or the ingestion of food.

4. The results in the guinea pigs are not striking or clear cut, due to their general susceptibility to the injected substances.

5. In the rabbit the maximum secretion from gastrin occurs in the first hour, from histamine in the second. This may be attributed to the preliminary vasoconstriction caused by histamine.

6. The pharmacologic action of histamine on the capillaries produces ideal conditions for secretion.

7. Histamine appears to have the property of exciting glandular tissue in general (pancreas, salivary gland and stomach).

8. The behavior of gastrin does not conform sufficiently to histamine to warrant the conclusion that they are the same substance.

9. Tyramine caused secretion in cats inconstantly, but failed in all other animals.

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GASTRIN STUDIES

IV. The Response of the Stomach Mucosa to Food and Gastrin Bodies as Influenced by Atropine

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The stimulating effect of morphine and pilocarpine (1) on gastric activity has been known for a number of years. The authors (2) have more recently extended Edkins' (3) observations on gastrin and have added to the list another substance, histamine, which when injected intramuscularly, causes gastric secretion. A large list of other substances has been reported by investigators at different times as capable of causing secretion. However, these have usually been administered orally and the evidence in favor of their activity is neither clear nor convincing. It seemed advisable to study the effect of atropine on the secretions caused by food and by gastrin bodies under which term for the present histamine is included. It was thought that such studies might enable us to estimate more accurately the value of these substances as stimulating agents and at the same time throw some light on the more fundamental problems of gastric secretion.

Effect of atropine on food stimulation. Riegel (4) has reported that atropine causes a decrease in the quantity of juice and in the height of acid after a standard meal of one liter of milk. He further showed that it decreased the secretion in cases clinically diagnosed as ulcers. From this and other studies into gastric motility as influenced by atropine and belladonna these bodies have assumed a definite value in the diagnosis of gastric ulcer and its management. We have substituted for Riegel's milk meal one which we hoped would give a maximum secretory effect, and with this have extended his observations. The meal consisted of 250 grams of lean meat, hashed through the meat chopper three times, 300 cc. of water and 5 grams of sodium chloride. The mixture was boiled long enough to change its appear-

ance to a uniform brown. Its stimulating power is shown in the protocols below.

Dog VIII. Pawlow stomach

Experiment 1. Fed standard diet. Hourly average for the first five hours was 12.7 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	*per cent*	*per cent*
Fifth hour	10.5	0.33	0.41
Sixth hour	3.0	0.24	0.38

Experiment 2. Fed standard diet. Hourly average for the first five hours was 16.0 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	*per cent*	*per cent*
Fifth hour	16.0	0.42	0.51
Sixth hour	6.0	0.29	0.40

Dog IX. Pawlow stomach with splanchnic nerves sectioned.

Experiment 1. Fed standard diet. Hourly average for the first five hours was 12.3 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	*per cent*	*per cent*
Fifth hour	13	0.44	0.50
Sixth hour	18	0.47	0.51

Experiment 2. Fed standard diet. Hourly average for the first five hours was 26.0 cc.

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL ACID
	cc.	*per cent*	*per cent*
Fifth hour	20	0.32	0.45
Sixth hour	11	0.30	0.37

We may conclude that normally the stomachs of these experimental animals displayed good secretory activity for at least six hours following the test meal used.

Effect of toxic doses of atropine on the secretion. In the first experiments a rather large dose of atropine was given which proved to be a

generally toxic one. It was only after twenty-four hours that the animals could be interested in food, while the effect on the pupil lasted for several days. The prostration with this dosage was profound.

Reference to table 1 shows that the effect on the quantity secretion is immediate, and the effect on the percentage of acidity occurs after thirty minutes. The dose was then reduced in order to see whether the inhibitory dose must be a generally toxic one. It was found that 1 mgm. of atropine sulphate (0.12 mgm. per kgm. body weight) would inhibit the secretion of the gastric juice over a period of five hours following the injection. No attempt was made to follow the secretion

TABLE 1

Effect of toxic dose of atropine on secretion from food stimulation

TIME INTERVALS	DOG VIII			DOG IX		
	Quantity	Hydrochloric acid		Quantity	Hydrochloric acid	
		Free	Total		Free	Total
Fed standard meal						
	cc.	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*
First hour	9.0	0.08	0.35	8.5	0.22	0.42
Second hour	18.0	0.34	0.49	16.0	0.36	0.49
Third hour	18.0	0.38	0.46	19.8	0.30	0.46
Injected 2 mgm. atropine sulphate per kilo body weight, intramuscularly						
Next half-hour	2.4	0.34	0.43	2.2	0.37	0.47
Next hour	0.7	0.00	0.10	0.4	0.00	0.29
Next hour	0.4	0.00	0.06	0.2	0.00	
Next hour	0.5	0.00	0.09	0.3	0.00	

longer and determine the exact time at which it would return. Such a period we took as evidence of definite and complete inhibition. This dose did not produce any other obvious symptoms. After the injections the animals lay down as usual, responded to petting and appeared normal in all respects, except for an occasional stretching of the mouth and licking of the nose. Hence we may say that an inhibitory dose need not be a generally toxic one.

Qualitative changes in the juice after atropine injections. By using still smaller doses one can analyze more clearly the steps in the inhibition. Tables 2 and 3 give the results of ten experiments carried out for this purpose. The first effect appears to be one on the quantity

secretion. Reference to tables 2 and 3 in which 0.05 and 0.025 mgm. doses were used shows that in all cases there is a sudden drop in the

TABLE 2

Effect of atropine on quantity partition of gastric juice after standard meal. Atropine sulphate injected intramuscularly

TIME INTERVALS	DOG VIII			DOG IX			DOG VIII		DOG IX	
	Quantities of juice			Quantities of juice			Quantities of juice		Quantities of juice	
One hour	6.0	7.0	3.0	13.0	9.5	13.5	4.0	5.0	2.0	3.5
One half-hour	10.0	7.0	3.0	12.0	9.0	10.0	4.6	2.7	2.5	8.4
	0.05 mgm. atropine sulphate			0.05 mgm. atropine sulphate			0.025 mgm. atropine sulphate			
One half-hour	3.3	1.8	1.7	3.0	2.0	3.4	2.5	2.0	1.0	3.8
One hour	4.0	5.2	2.2	6.8	9.0	11.0	2.9	2.2	1.5	8.3
One hour	6.0	7.8	2.8	19.0	17.1	17.4	2.5	3.8	3.9	11.5
One hour		6.9	2.6		18.7	14.4	2.5	4.3	9.5	11.0

TABLE 3

Effect of atropine on total acid partition in the gastric juice after standard meal

TIME INTERVALS	DOG VIII			DOG IX			DOG VIII		DOG IX	
	Per cent total hydrochloric acid*			Per cent total hydrochloric acid			Per cent total hydrochloric acid		Per cent total hydrochloric acid	
One hour	0.48	0.45	0.36	0.55	0.51	0.55	0.34	0.36	0.20	0.55
One half-hour	0.48	0.51	0.48	0.58	0.57	0.58	0.46	0.48	0.40	0.58
	0.05 mgm. atropine sulphate			0.05 mgm. atropine sulphate			0.025 mgm. atropine sulphate			
One half-hour	0.47	0.51	0.46	0.54	0.58	0.58	0.45	0.43	0.33	0.55
One hour	0.36	0.41	0.27	0.46	0.46	0.51	0.37	0.36	0.36	0.51
One hour	0.43	0.45	0.35	0.51	0.55	0.64	0.31	0.42	0.50	0.56
One hour		0.46	0.33		0.57	0.58	0.27		0.50	0.55
One hour		0.42			0.58	0.58				

* Since the free acid curve figures followed so closely those of total acid they are omitted for the sake of brevity.

quantity output. This comes on in the first half-hour and with these doses persists for one and one-half to two hours. The acidity (table 3)

is only slightly reduced. Indeed one may consider the acidity changes well within the limits of variation of normal stomachs on a test meal. However if the dose of atropine be increased to 0.1 to 0.2 mgm. then the drop in acid is unmistakable.

These facts are quite clearly illustrated in the attached protocol.

Dog VIII. Pawlow stomach

TIME	QUANTITY	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	*per cent*	*per cent*
10:20	Fed the standard meal		
11:20	15.5	0.46	0.49
12:20	17.0	0.53	0.55
12:24	Injected 0.2 mgm. atropine sulphate intramuscularly		
12:54	1.6	0.48	0.55
1:24	0.4	0.27	0.38
2:24	1.2	0.04	0.07
3:24	1.4	0.00	0.02
4:24	1.0	0.12	0.21

It is clear that a 50 to 80 per cent reduction in the quantity output may be made without affecting the acid concentration. When, however, the reduction is greater than this, then changes in the acid are noted.

Pepsin. If we measure the pepsin content by Mett's tubes and express the concentration in the square of the millimeter of albumin digested by the juice diluted to twice the original volume in 0.3 per cent HCl, then the following table (table 4) shows the immediate and definite effect of the intramuscular injection of such small doses as 0.05 to 0.025 mgm. atropine sulphate.

In a normally secreting stomach as the quantity of juice is increased the concentration of peptic units falls. Thus in dog VIII, the quantity rose from 4 cc. per hour to 9.2 cc. per hour, while the pepsin concentration expressed in squares of millimeters of albumin digested in Mett's tubes fell from 12.25 to 2.56. In the case of dog IX, the quantity rose from 3.5 cc. to 16.9 cc. while the concentration fell from 5.76 to 2.72. At the close of a period of normal digestion, the reverse of this occurs; the quantity drops and the concentration per cubic centimeter rises. This condition is in sharp contrast to that occurring after the injection of atropine. Here both the quantity of gastric juice and the pepsin concentration show an immediate reduction. In other words, we have a definite inhibition or toxic action on the secretory mechanism. We

may then place the secretion of pepsin along with that of the quantity, and say that when these two are affected the mechanism has received a first degree or first level damage. When the acid is lowered, the damage is of the second degree or to a second lower level.

Effect of atropine on gastrin stimulation.[1] In studying this phase of the problem an attempt was made to secure a dose of gastrin which would throw the gastric secretory mechanism into a uniform rate of activity, and then give increasing doses of atropine.

The stimulating dose of gastrin chosen was 0.25 cc. of Sb6B. This was a partly purified preparation obtained by removing proteins and other inert substances by alcohol and basic lead acetate precipitations.

TABLE 4

Effect of atropine on pepsin content under food stimulation

BEFORE	DOG VIII			DOG IX		
One hour	12.25	4.41	4.42	6.76	5.76	10.89
Second hour	2.56	1.21			2.72	
Atropine dosage	0.025 mgm.		0.05 mgm.	0.025 mgm.		0.05 mgm.
One to two hours	1.64	0.12	1.0	4.84	3.06	5.76
Three to four hours	1.21	0.06	1.21	2.96	1.69	14.44
	8.51	1.44		4.41	1.69	16.81
				6.25	1.69	

Note the return of peptic content to normal as the effect of the atropine wears off.

The gastrin was given three-fourths to one hour after the atropine. Both the atropine and the gastrin were injected intramuscularly. The results are shown in table 5.

The animal began to show signs of toxicity with 0.8 mgm. atropine but took food at the end of the experiment. It will be noted that the secretion was inhibited quite as much by the 0.2 as 0.8 mgm. and that it took a generally toxic dose, 2.4 mgm., practically to abolish the secretion.

[1] Since completing this work our attention was called to an article by Maydell (Dissert. Kiev, 1917, 170) in which according to the reviewer "atropine diminishes the secretory effect of gastric secretin" (cited from Physiological Abstracts, 1917–18, II, 146. Abstr. no. 1700).

TABLE 5

Effect of increasing doses of atropine on gastrin stimulation

ATROPINE SULPHATE	GASTRIN	TIME	QUANTITY OF JUICE	HYDROCHLORIC ACID	
				Free	Total
mgm.	*cc.*		*cc.*	*per cent*	*per cent*
None	0.25	1st hour	3.0	0.23	0.26
		2nd hour	0.25	0.22	0.26
0.2	0.25	1st hour	1.8	0.25	0.27
		2nd hour	0.5	0.00	0.09
0.8	0.25	1st hour	1.7	0.26	0.30
2.4	0.25	1st hour	0.6	0.18	0.22
		2nd hour	0.2		

It was next of interest to know the effect on the secretion of increasing the dose of gastrin. The protocols of two experiments follow.

Dog III. Pawlow stomach

TIME	JUICE	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	Control experiment		
	cc.	*per cent*	*per cent*
8:50	Dressed		
9:50	0.3	0.00	trace
9:50	1 cc. gastrin (Sb6B) intramuscularly (4 times the minimal secretory dose)		
10:50	6.2	0.28	0.37
11:50	8.8	0.46	0.48
12:35	4.1	0.43	0.46
	Atropine experiment on the same dog		
10:10	2.4 mgm. atropine sulphate intramuscularly		
11:10	1 cc. gastrin (Sb6B) intramuscularly		
12:10	3.5	0.23	0.27
1:10	5.3	0.41	0.47
2:10	2.4	0.38	0.41

The dose of atropine, 2.4 mgm., which had completely inhibited the secretion from 0.25 cc. of gastrin has depressed the secretion only (40 per cent) when the dose of gastrin was quadrupled. Duplicate

experiments were run on dogs IV and V with striking confirmation of the findings expressed above.

Working in the ranges of toxic doses the following experiments are of interest as showing the quantitative antagonistic relations of the two preparations.

Effect of atropine on histamine stimulation. The quantitative antagonism of the gastrin series also holds for histamine and atropine. This is beautifully illustrated in table 7 below.

The question natually arises whether it is possible, by giving doses of atropine sufficiently toxic, to render the gastric mechanism insensitive to histamine stimulation. For this two gastric fistulae animals were selected since a qualitative rather than an absolute quantitative result was desired. The protocols of experiments in which 20 to 21 mgm. of atropine sulphate (approximately 3 mgm. per kilo body weight) were administered are appended. In these an unquestionable secretion is present, although it is reduced in quantity.

Dog VII. Gastric fistula

TIME	JUICE	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	*per cent*	*per cent*
9:45	Dressed		
10:15	2.0	0.03	0.01
11:15	3.0	0.00	0.01
11:30	Injected 20 mgm. atropine sulphate (intramuscularly)		
12:00	2.0	0.00	0.03
12:00	Injected 0.5 mgm. histamine hydrochloride (intramuscularly)		
12:30	12.8	0.38	0.46
1:00	7.6	0.46	0.53
1:40	0.5	Present not determined	

Two other experiments on the same animal yielded similar results. Dog VI (gastric fistula) also gave definite secretion after similar generally toxic doses of atropine (3.5 mgm. per kilo body weight). It is further interesting to note that the histamine always increased the general toxic symptoms already produced in the animal by the atropine, rather than neutralizing them.

Relative efficacy of food and histamine stimulation. In a previous section it has been shown that a food stimulation can be completely inhibited by 1 mgm. of atropine, and yet we have just seen that 21 mgm. of atropine were not sufficient to inhibit the secretion from 0.5 mgm. of histamine. In order to test this relationship more clearly an

TABLE 6

Effect of atropine on the stimulation produced by the intramuscular injection of 1 cc. gastrin (Sb6B). Dog IV—Pawlow stomach. The gastrin was injected three-fourths to one hour after the atropine.

ATROPINE SULPHATE	GASTRIN	TIME	JUICE	HYDROCHLORIC ACID	
				Free	Total
mgm.	*cc.*		*cc.*	*per cent*	*per cent*
None	1	1st hour	10.1	0.32	0.36
		2nd hour	2.6	0.27	0.36
2.4	1	1st hour	3.5	0.27	0.32
		2nd hour	2.3	0.32	0.36
3.2	1	1st hour	2.9	0.27	0.32
		2nd hour	0.5	0.20	0.26
4	1	1st hour	3.0	0.16	0.23
		2nd hour	1.8	0.32	0.36

TABLE 7

Effect of atropine sulphate on stimulation by 0.5 mgm. histamine hydrochloride both injected intramuscularly

ATROPINE SULPHATE	HISTAMINE HYDROCHLORIC ACID	TIME	DOG VIII. PAWLOW STOMACH			DOG IX. PAWLOW STOMACH		
			Juice	Hydrochloric acid		Juice	Hydrochloric acid	
				Free	Total		Free	Total
mgm.	*mgm.*			*per cent*	*per cent*		*per cent*	*per cent*
None	0.5	1st hour	10.5	0.27	0.38	21.8	0.56	0.60
		2nd hour	1.0	0.15	0.22	2.6	0.37	0.41
0.025	0.5	1st hour	6.0	0.36	0.44	16.2	0.48	0.58
		2nd hour	0.6	0.04	0.01	2.2	0.29	0.41
0.05	0.5	1st hour	0.5	0.27	0.33	10.0	0.38	0.42
		2nd hour	0.1			1.0	0.36	0.40
0.1	0.5	1st hour	2.0	0.18	0.32	7.0	0.37	0.50
		2nd hour	1.0	0.25	0.30	0.2		
0.2	0.5	1st hour	2.1	0.19	0.23	6.5	0.41	0.46
		2nd hour	0.5	0.23	0.31	0.6	0.44	0.48

experiment was run in which the secretion induced by food was inhibited by atropine and then started by histamine.

Dog VIII. Pawlow stomach

TIME	JUICE	FREE HYDROCHLORIC ACID	TOTAL HYDROCHLORIC ACID
	cc.	*per cent*	*per cent*
9:50	Dressed		
10:50	1.4		
11:00	Ate standard meal		
12:00	2.0	0.00	0.07
1:00	13.0	0.30	0.36
1:10	Injected 0.5 mgm. atropine sulphate (intramuscularly)		
1:40	1.5	0.23	0.30
2:10	0.4	Trace not estimated	
2:15	Injected 0.5 mgm. histamine hydrochloride (intramuscularly)		
2:45	7.8	0.43	0.47
3:25	3.8	0.44	0.48
3:45	2.2	0.39	0.42
4:30	4.0	0.40	0.45

An experiment run on the same animal two days later showed the period of inhibition for 0.5 mgm. of atropine sulphate under food stimulation to be approximately three hours and other experiments have shown that injections of histamine hydrochloride falling on an empty stomach rarely produce secretion for a period of over one and one-half hours. With these facts before us the examination of the protocol above shows that the atropine inhibited the food secretion, that the histamine stimulated the mechanism immediately, in its usual fashion, and that just as the results of the latter stimulation came to a standstill the food secretion reasserted itself.

DISCUSSION

Qualitative changes in gastric juice under atropine injection. Our findings on the effect of atropine on secretion under food stimulation agree well with those of Riegel (4) for the stomach and of Bylina (5) for the pancreas. The latter has studied the question more completely and he finds a reduction in quantity of juice, acid, total nitrogen, enzyme and dried residue. Our results show that the pepsin and quantity output are first affected. The concentration of acid may be maintained until the quantity has been reduced 50 per cent to 80 per cent, after which it in turn rapidly decreases. Attention has already

been called to the observation that with rapid increase in the quantity of juice secreted by a normal stomach the acid keeps pace and is able to maintain itself at a high concentration. On the other hand the pepsin partition lags behind to such an extent that its concentration in reality becomes quite low. Further, one of the authors (6) has previously shown that in parathyroid tetany a juice of fairly high acid content and extremely low peptic activity is frequently secreted. All of these observations suggest that the mechanisms of the acid and pepsin secretion are not the same, and that under different states of the gastric mucosa, which may occur in the course of a normal digestion period or be induced experimentally, these mechanisms may respond differently to the same stimulus.

Site of action of atropine and gastrin bodies. A quantitative antagonistic relation appears to exist between atropine on one hand and gastrin and histamine on the other. With a fixed small dose of gastrin or histamine the secretion can be practically inhibited by raising the dose of atropine. However, generally toxic doses of atropine which are short of the lethal point do not completely inhibit secretion when the gastrin dosage is increased. Secretion can therefore always be obtained by these bodies after atropine.

Certain generalizations have grown out of the use of atropine in the study of secretory mechanisms. One generalization is to the effect that atropine inhibits secretion originating from nervous mechanisms, but not that from a chemical or humoral origin. In the case of the salivary glands it has been shown that after atropine the chorda tympani is no longer able to excite secretion, but sympathetic stimulation usually causes a flow of saliva. This localizes the action of atropine to the terminations of the chorda lingual nerve. These studies were, however, carried out under ether anesthesia.

Bayliss and Starling (7) state that it is impossible to paralyze the flow of pancreatic juice produced by secretin by any dose of atropine although they do not specifically state what dosages were used. They, too, worked under ether anesthesia.

Bylina (5) found that 0.005 gram atropine reduced, but did not abolish the flow of pancreatic juice obtained by introducing HCl into a duodenal fistula, and neutral oil and sodium oleate into stomach. In other experiments he drained the gastric juice from a fistula so that none was escaping into the duodenum and found a spontaneous fasting secretion of the pancreas which was changed and reduced, but not abolished by a similar dose of atropine. He reviews the literature

rather carefully and emphasizes the necessity of working with chronic fistulae and without the use of ether. He interprets all these findings to mean that the nervous element in secretion is involved to the extent to which a reduction in secretion has occurred.

Von Anrep (8) working with decerebrated dogs found that atropine paralyzes the secretory but not the inhibitory fibers in the vagus going to the pancreas. This effect is not a vascular one, for careful measurement of blood flow through the gland shows that the vagi carry no vasomotor fibers. The inhibitory fibers he believes are really motor fibers to the ducts which analogy suggests should be affected by the atropine also.

Mathews (9) studying the secretion of saliva after clamping the blood supply to the gland emphasized the view that the action of atropine is rather on the cell than on the terminations in the chorda tympani. He presented no conclusive evidence supporting this view, but insisted its adoption was a more plausible explanation of the facts in hand.

Popielski (10) observed that the decreased coagulability of the blood drawn from the vena sub-maxillaris interna as brought about by stimulation of the chorda tympani was again increased in coagulability by the injection of 15 cc. of 0.1 per cent atropine sulphate solution. Further, that if at the time of the most marked atropine effect the chorda is again stimulated the increased coagulability remains and we have no flow of saliva. Similarly he found atropine to increase the coagulability of blood rendered less coagulable by the injection of barium chloride or pilocarpine. However the decreased coagulability and the secretion of saliva brought about by stimulation of the sympathetic nerves to the salivary gland is not overcome by atropine.

We certainly are far from knowing the site of action of atropine. The whole subject needs reinvestigation. We can at least say that while the terminations of the vagi may be affected by atropine they are not the sole site upon which the drug is acting in reducing the secretion under histamine or gastrin stimulation. Our previous studies have shown that doubly vagotomized animals give excellent secretion following the injection of these substances. There remains to be considered the nervous element in Auerbach's plexus and the cell itself. So far as histamine and gastrin are concerned the decrease in secretion is a uniform reciprocal of the dose of atropine. The curve is a uniform one with no irregularities. The food stimulation is very readily inhibited by atropine but subsequent injections of histamine showed

the mechanism to be still quite sensitive to this excitant. Further, there appears to be one irregularity, in its curve of decrease; the acid concentration is well maintained after the quantity and peptic partitions have suffered seriously.

We therefore suggest that in the ranges within which atropine paralyzes the food secretion, its point of action is largely nervous (terminations of vagi and Auerbach's plexus); in larger doses (1 mgm.) of atropine sulphate its action must be largely on the gland cells. If the physiological secretion of juice is in any way dependent on substances such as histamine and gastrin, then we would suggest that these bodies may be liberated under nervous influences. Once liberated they become the effective agent in arousing the gastric cell directly to activity. Take as an illustration dog IX which secreted 15 to 18 cc. of juice an hour on a standard meal, a secretion which was inhibited completely by 0.5 mgm. of atropine. The same animal secreted 21 cc. of juice on 0.5 mgm. of histamine. After the same dose of atropine (0.5 mgm.) the quantity still amounted to 14 cc. per hour. It is easy to see how the secretion might be blocked at the site of the nerves with their lower threshold of sensitivity to atropine.

The general toxic effect of atropine may be expressed in nervous depression or exaltation, restlessness and vomiting. All of these symptoms indicate that the atropine effect is now on the general body cells. Toward this general poisoning the gastric cells appear to be neither more sensitive nor more resistant. The evidence of their poisoning is found in their progressive failure to respond to stimulation. Thus the lethal dose (for the animal) of atropine becomes the one of complete inhibition for gastrin bodies. This conforms with our findings that an inhibitory dose to food stimulation is not a generally toxic one. However, when once these chemical excitants (gastrin bodies) are liberated then only a generally toxic dose of atropine, which abolishes not only the secretion of the gastric glands but also modifies all other protoplasmic activities, must be used before the secretion can be arrested.

If this view should prove correct we have the analogy with the pancreas as follows: Pancreatic secretin originates in the duodenal mucosa at a point where the effective stimulus (HCl) is normally present. Much discussion has centered about what part the nerves here play in the liberation of secretin from pro-secretin. The blood must now transfer the secretin to the pancreas. In the case of the stomach the whole mechanism is condensed within the mucosa of the stomach

for here the effective stimulus, whatever it be, can liberate the gastrin rendering it immediately available for secretory stimulation. At this point attention is called to the fact that through a rather extended series of experiments (2) we have never been able to find any gastrin bodies in the gastric juice, which is in keeping with this view.

Finally, it is evident that two such classes of drugs as histamine and gastrin on the one hand and atropine on the other have no power of neutralizing each other. The effect of combined doses is to increase rather than to diminish the toxicity on the general cells of the body using the nervous system as an index. In small doses they are possibly acting upon the secretory structures at different sites. In larger doses the antagonism is transferred to the gland cell, whose response or inhibition during viability is determined by the concentrations of atropine and gastrin bodies presented to the cell at any given unit of time.

CONCLUSIONS

1. A stimulation of the gastric glands with food is completely inhibited in the dog over a period of more than five hours by the intramuscular injection of 1 mgm. of atropine sulphate. This inhibitory dose is not a generally toxic one.

2. The quantity and pepsin concentration are immediately and progressively reduced by doses of atropine sulphate as small as 0.025 mgm. The acid concentration is not affected until the quantity has been reduced by an amount approximately 50 to 80 per cent of its original value.

3. Gastrin and histamine on the one hand, and atropine on the other, have a quantitative antagonistic effect on gastric secretion. By increasing the dose in either class of substances a secretion or inhibition can be obtained.

4. Gastrin and histamine stimulation occur following injections of quantities of atropine which appear to be just short of the lethal dose.

5. The antagonism does not consist of a chemical neutralization since each substance increases the general toxicity of the other for body cells if the nervous system be taken as an index.

6. The view is favored that in small doses atropine and gastrin bodies attack the secretory mechanism at different points; in larger doses the site of their action is transferred to the gland cell.

7. If gastrin and related bodies are of physiological importance it is suggested that they may be liberated by secretory nerves and that they then become the active exciting agent of the cell to secretion.

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FURTHER OBSERVATIONS ON THE RELATION OF THE SPINAL CORD TO THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS, AND THE ACTION OF STRYCHNINE AFTER CERVICAL CORD SECTION

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In a previous paper (1) we concluded that in the cat after transection of the spinal cord in the cervical region the epinephrin output is not abolished, but is still sustained from the upper thoracic cord. In acute experiments it was shown that the previous rate of output might continue unchanged after the cervical cord transection, indicating that the mechanism in the thoracic cord was actually functioning at the moment of the cervical section. It is obvious that if there is an epinephrin-secretory center in the thoracic cord it may be subject to spinal shock after lesions of the cervical cord like other spinal centers, and that the susceptibility to shock might be expected to vary in different kinds of animals and might be influenced by the experimental conditions, especially the circulatory changes caused by the cervical lesion. In the present research, accordingly, we have employed not only cats, but also dogs and one or two monkeys and rabbits, and have eliminated the upper portions of the central nervous system in cats by ligation of their arterial supply as well as by the knife. The stimulating action of strychnine upon the central mechanisms governing the epinephrin output having become known to us since our last paper, we have made a number of experiments in which this drug was used after the cervical transection, in order to see whether the epinephrin output was increased by it, as is the case when the nervous system is intact and the epinephrin being liberated at the ordinary rate.

Note. By "ordinary output" or "normal output" we mean an output within the range observed by us in animals in which we have not purposely used procedures calculated to alter the output (such as the administration of drugs like

strychnine or artificial stimulation of the splanchnic nerves). In referring to this normal output we often use the phrase "under our experimental conditions," or words to that effect. If we sometimes omit this qualifying phrase it is simply because its continual repetition becomes tedious. It is quite true that as we have gone on and have seen within how relatively narrow a range the output, as measured by us, varies with different anesthetics and different operations, we have inevitably tended more to the opinion that a quantity which is not demonstrably affected by changes in such important experimental conditions must depend upon something more fundamental than the anesthesia, opening of the abdomen, etc. The fact that the output in one and the same acute experiment is sensibly the same when adrenal blood is first obtained as it is later on when additional operative procedures have been employed, suggests that what we term the ordinary or normal spontaneous output is not initiated or sustained by the trauma. Cannon (2) has affirmed that there is no doubt that opening the abdominal cavity under anesthesia results in a discharge of impulses along the splanchnic fibers governing the adrenal secretion. "The adrenal glands, therefore, are continuously and abnormally stimulated if the abdomen is opened." The only evidence he offers for this is the statement that opening the abdomen causes impulses to descend the splanchnic fibers to the intestines which inhibit their movements. As the adrenal secretory fibers are also sympathetic and run in the splanchnics they must be stimulated too. It would be just as logical to say that as vasoconstrictor fibers for the splanchnic area are sympathetic and run in the splanchnics they must also be stimulated and therefore there should be a marked rise of blood pressure due to splanchnic vasoconstriction on opening the abdomen. The only way of showing that opening the abdomen increases the epinephrin output is to prove it experimentally and this has not been done. The same is true of the alleged influence of anesthetics. Cannon says "evidence exists in the inhibitory influence of anesthesia on gastro-intestinal movements that anesthesia alone can arouse splanchnic impulses." And among these he obviously includes impulses passing along the splanchnics to the adrenals to increase the epinephrin output. Yet he has stated in another paper (3) that he has found anesthesia by urethane to be "in fact unattended by any increase of adrenal secretion demonstrable by our tests." Now if urethane does not increase the output, ether and morphine certainly cannot do so since we have found the output with these anesthetics less if anything on the average than with urethane. While we do not think that Cannon's work has shown anything at all as to the influence or want of influence of urethane upon the epinephrin output, and he has published no experimental data whatever upon this point, his conclusion that the employment of an anesthetic has no influence upon the results of his own experiments whereas the employment of the same anesthetic vitiates ours, is somewhat puzzling. But not only can he use urethane with impunity, while he indicates that we cannot do so, but he can open the abdomen, of course under anesthesia, and still obtain a certain reaction with asphyxia which he interprets as proving an increased output of epinephrin, whereas he says that when we open the abdomen we immediately deprive ourselves of all possibility of obtaining evidence of any such effect since we are working with an animal under an anesthetic and with opened abdomen. Cannon's suggestion that the reason we fail to obtain evidence of an increased output of epinephrin in such conditions as asphyxia is that the opening of the abdomen produces a secretion which

is "unsurpassable." This suggestion is inconsistent with his own statements that the output of epinephrin caused by stimulation of the central end of the sciatic is "from 5 to 25 times the amount regarded by Stewart and Rogoff as the normal output," and that the reaction on which he relies to demonstrate the increased output is elicited in equal intensity by asphyxia.

The results of the greater part of the experiments are condensed into three tables. Only a sufficient number of protocols to illustrate the various types of experiment or to illustrate experiments which could not be tabulated have been introduced.

ACUTE EXPERIMENTS ON CATS

In table 1 are shown the results of acute experiments on cats in which the epinephrin output was assayed on rabbit intestine (and uterus) segments before and after transection of the cervical cord.

In three of the cats (350, 407 and 67) the output, which was of the ordinary magnitude before the cord section, remained practically unaltered by that operation. The data on one of these animals have already been published (1), but for the sake of completeness they are included in the table. In one of these cats (407) the intravenous injection of strychnine after transection of the cord raised the output of epinephrin to seven times the initial value. As the experiment illustrates at the same time the possibility of dividing the cervical cord without reducing the epinephrin output and without interfering with the characteristic action of strychnine upon the output, the condensed protocol is reproduced.

Condensed protocol. Cat 407; female; weight, 2.46 kgm.

9.05 a.m. Anesthetized with ether, inserted cannulae into trachea, carotid artery and external jugular vein. Obtained a specimen of indifferent (jugular) blood.

9.20 a.m. Exposed cord below mid-cervical region. Made cava pocket, tying coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.

9.45 a.m. Cava pocket completed. Collected adrenal blood.

9.48½ a.m. First specimen, 1.9 gram in 30 seconds (3.8 grams per minute).

9.49 a.m. Second specimen, 4.85 grams in 90 seconds (3.2 grams per minute).

9.55 a.m. Blood pressure 103 mm. of mercury.

10.00 a.m. Transected cord above the origins of the 6th pair of cervical nerves (position of section was verified at autopsy). Spontaneous respiration continued; artificial respiration not employed. After cord section the pupils were about one-third dilated and eye reflexes good.

10.03 a.m. Blood pressure 50 mm. of mercury.
10.05 a.m. Third adrenal specimen, collected for 30 seconds (not weighed).
10.05½ a.m. Fourth adrenal specimen, 1.85 gram in 5 minutes (0.37 gram per minute).
10.10 a.m. Started artificial respiration.
10.15 a.m. Blood pressure 50 mm. of mercury.
10.17 a.m. Intravenous injection of 0.45 mgm. strychnine: within ½ minute after strychnine injection a tonic spasm occurred for a few seconds and this was followed by clonic convulsions which recurred intermittently during the rest of the experiment.
10.20 a.m. Fifth adrenal specimen, collected for 30 seconds (not weighed).
10.20½ a.m. Sixth adrenal specimen, 2.3 grams in 10 minutes (0.23 gram per minute).
10.33 a.m. Blood pressure 30 mm. of mercury. Another specimen of indifferent blood was now obtained. Combined weight of adrenals, 0.375 gram. (There was about 75 per cent of serum in the bloods).

As it is impossible to reproduce all of the tracings used for the assay even in one experiment, we shall illustrate this one typical experiment more completely than the rest, for which it must serve as a sample of the manner in which we arrive at the epinephrin concentrations. Some of the tracings are reproduced in figures 1 to 4.

The 2nd specimen was found to be decidedly weaker than 1:4,000,000 adrenalin (fig. 1, confirmed by other observations), decidedly stronger than 1:8,000,000, somewhat stronger than 1:6,660,000, somewhat weaker than 1:5,300,000. It was taken at 1:6,000,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00021 mgm. per kgm. per minute.

The 4th specimen, collected after section of the cord, was much stronger than 1:1,300,000 (fig. 2), not very different from 1:660,000 (fig. 3). Another observation indicated that it was probably somewhat weaker than 1:660,000. It was distinctly weaker than 1:530,000 (fig. 3). It was taken at 1:700,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00021 mgm. per kgm. per minute.

The concentration of epinephrin in the 6th adrenal specimen, obtained after injection of strychnine, was so great that in order to make a good assay it was necessary to dilute it with indifferent blood. In this way it was shown to be much stronger than 1:165,000 adrenalin (fig. 4). The 6th specimen diluted with 3 volumes of indifferent blood was much stronger than 1:660,000 adrenalin. Diluted with 7 volumes of indifferent blood it was still found to be much stronger than 1:1,000,000, i.e., the 6th specimen was much stronger than 1:125,000.

TABLE 1

Acute experiments. Cats

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD							REMARKS
	Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		
									For animal	Per kilogram						For animal	Per kilogram	
			gms.	*gms.*	*sec.*	*gms.*	*mm. of mercury*		*mgm.*	*mgm.*	*gms.*	*sec.*	*gms.*	*mm. of mercury*		*mgm.*	*mgm.*	
320	3.15	v–vi	0.295	3.25	300	0.65	40	1:6,000,000	0.00011	0.000035	4.4	330	0.8	44	1:6,000,000	0.00013	0.00004	Shock on cord exposure: 80 cc. Ringer injected before collection of each adrenal specimen.
321	3.89	above iv	0.922	2.75	360	0.46		1:2,700,000	0.00017	0.000045	2.05	720	0.17		1:1,000,000	0.00017	0.000045	Without injection of Ringer
											2.55	360	0.425		1:2,700,000	0.00016	0.00004	With Ringer injection, 100 cc.
322	2.25	v–vi	0.487	7.0	120	3.3	144	1:4,500,000	0.0007	0.0003	3.4	720	0.30	58	1:800,000	0.0004	0.00017	
											1.3	600	0.13	37	1:500,000	0.00026	0.00011	
384	2.63	above vi	0.275	4.95	120	2.5	117	1:3,500,000	0.0007	0.00027	7.1	90	4.7	46	1:9,400,000	0.0005	0.0002	After cord section the blood pressure fell to 32 mm. of mercury. 100 cc. Ringer injected and circulation improved.

399	2.69	v–vi	0.268	5.3	90	3.5	110	1:5,000,000	0.00065	0.00025	1.5	330	0.3	47	1:1,500,000	0.0002	0.000075	
											1.8	240	0.45	40	1:250,000	0.0018	0.0007	0.5 mgm. strychnine injected before collection of this specimen.
406	2.09	vi–vii	0.474	3.75	120	1.87	112	1:4,600,000	0.0004	0.00024	3.15	120	1.57	86	1:6,000,000	0.00025	0.00012	After cord section blood pressure fell to 26 mm. 60 cc. Ringer injected and blood pressure rose to 86 mm. of mercury.
											1.15	360	0.2	40	1:150,000	0.0013	0.0006	0.6 mgm. strychnine injected before this specimen.
407	2.46	above vi	0.375	4.85	90	3.2	103	1:6,000,000	0.00053	0.00021	1.85	300	0.37	50	1:700,000	0.00053	0.00021	
											2.3	600	0.23	30	1:70,000	0.0033	0.0014	0.45mgm. strychnine injected before this specimen.
418	1.96	v	0.232	(a)3.2	90	2.1	105	1:4,000,000	0.0005	0.00025								
				(b)3.35	120	1.7	94	1:1,700,000	0.001	0.0005	1.0	600	0.1	24	1:100,000	0.001	0.0005	0.25 mgm. strychnine injected after (a). Blood pressure rose to 120 mm. Hg. Cord section after strychnine injection.
67	2.31	above v	0.440	10.0	120	5.0		1:13,000,000	0.0004	0.00017	2.1	430	0.3		1:800,000	0.0004	0.00017	

TABLE 1—*Concluded*

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD							REMARKS
	Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		
									For animal	Per kilogram						For animal	Per kilogram	
			gms.	*gms.*	*sec.*	*gms.*	*mm. of mercury*		*mgm.*	*mgm.*	*gms.*	*sec.*	*gms.*	*mm. of mercury*		*mgm.*	*mgm.*	
324	2.1		0.367	5.55	180	1.85		1:9,000,000	0.0002	0.0001	4.75	300	0.95		1:4,000,000	0.00024	0.0001	Bulb and brain eliminated by ligation of head arteries.
											4.85	240	1.21			0.00024	0.0001	
32	2.05		0.252	5.15	180	1.72		1:2,500,000	0.0007	0.00035	2.3	300	0.5		1:675,000	0.0007	0.00035	Bulb and brain eliminated by ligation of head arteries.
435	2 5		0.314	1.9	150	0.76		1:800,000	0.00095	0.00038	2.5	180	0.83		1:700,000	0.0012	0.00048	Bulb and brain eliminated by ligation of head arteries.
											1.65	360	0.275		1:450,000	0.00061	0.00024	
383*	3.98	v–vi	0.602	8.05	90	5.4	129	1:5,400,000	0.001	0.00025	4.45	180	1.48	93	1:3,000,000	0.0005	0.00013	Both adrenals discharging in all specimens.
385*	2.75	iv	0.612	6.2	180	2.06	114	1:2,900,000	0.0007	0.00025	3.25	240	0.81	40	1:1,000,000	0.0008	0.0003	Right adrenal only discharging in both specimens.

* In these two animals the spinal operation was a semisection, left in 383, and right in 385.

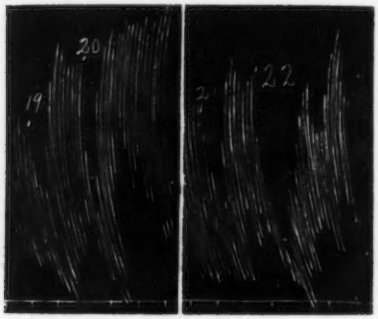

Fig. 1. Intestine tracings. Bloods from cat 407. At *19* and *21* Ringer was replaced by indifferent (venous) blood and this at *20* by the 2nd adrenal specimen (collected before cervical cord section); at *22* by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). As in all the figures the time trace is in half-minutes. Reduced to one-half.

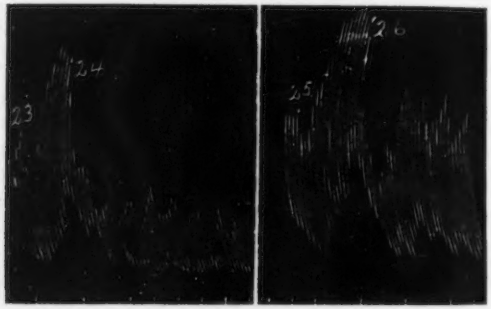

Fig. 2. Intestine tracings. Bloods from cat 407. At *23* and *25* Ringer was replaced by indifferent (venous) blood and this at *24* by the 4th adrenal specimen (collected 5½ minutes after cervical cord section); at *26* by indifferent blood to which was added adrenalin to make a concentration of 1:1,300,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-sevenths.

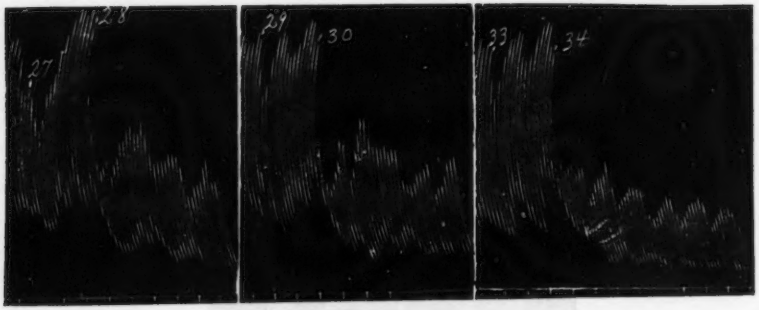

Fig. 3. Intestine tracings. Bloods from cat 407. At *27*, *29* and *33* Ringer was replaced by indifferent (venous) blood and this at *28* by indifferent blood to which was added adrenalin to make a concentration of 1: 660,000; at *30* by the 4th adrenal specimen (collected 5½ minutes after cervical cord section); at *34* by indifferent blood to which was added adrenalin to make a concentration of 1: 530,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-sevenths.

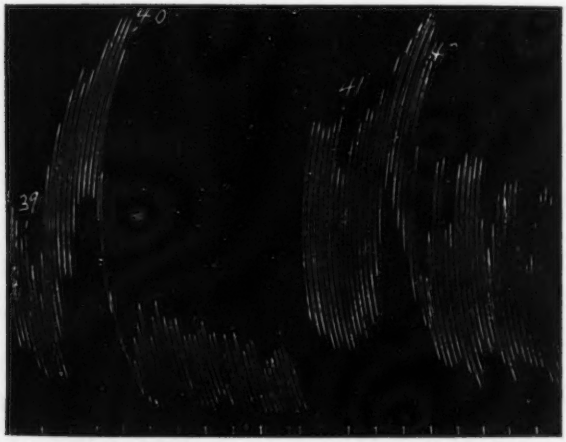

Fig. 4. Intestine tracings. Bloods from cat 407. At *39* and *41* Ringer was replaced by indifferent (arterial) blood and this at *40* by the 6th adrenal specimen (collected 3 minutes after intravenous injection of strychnine and 10 minutes after cervical cord section) diluted with 3 volumes of indifferent blood; at *42* by indifferent blood to which was added adrenalin to make a concentration of 1: 660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

In the same way it was shown that the specimen did not differ much from 1:66,000, being probably a little weaker. This was confirmed by numerous observations on the adrenal blood diluted with 7 volumes of indifferent blood, and also on the adrenal blood diluted with 14 volumes of indifferent blood. The 6th specimen was finally taken at 1:70,000, corresponding to an output of 0.0033 mgm. per minute for the cat, or 0.0014 mgm. per kgm. per minute.

In two of the cats in table 1 (320 and 321) the output was already much below the ordinary average before the cord was cut. These were two of the earliest experiments, and at this time we were in the habit of passing a thread under the cord after exposure. The thread was not tied, of course, but was used to make sure that the transection was complete. Later on the section was made intradurally through a longitudinal slit in the dura by means of small blunt pointed scissors, and the cord was not disturbed before collection of the initial adrenal specimen, apart from being laid bare at the level where it was to be divided. Blood vessels lying between the cord and the walls of the vertebral canal escaped injury except, of course, where the bone was removed. In cats 320 and 321 the low initial output of epinephrin was associated with a low blood pressure. Transection of the cord left the output unaltered. The condensed protocol of cat 321 follows.

Condensed protocol. Cat 321; male; weight, 3.89 kgm.

8.30 a.m.	Urethane, 6 grams per stomach tube.
9.00 a.m.	Inserted tracheal cannula, exposed cord in mid-cervical region and placed a loose ligature under the cord. During the manipulations around the cord the cat stopped breathing and the circulation became feeble. Artificial respiration was at once started. Obtained a specimen of indifferent blood (femoral vein). Made cava pocket.
10.06 a.m.	Pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above the bifurcation) were tied in addition to the veins entering the cava pocket. Collected adrenal blood.
10.10 a.m.	First specimen, 0.9 gram in 1 minute.
10.11 a.m.	Second specimen, 2.75 grams in 6 minutes (0.46 gram per minute).
10.20 a.m.	Transected cord just above the origins of the 4th pair of cervical nerves.
10.23 a.m.	Third adrenal specimen collected for 1 minute (not weighed).
10.24 a.m.	Fourth adrenal specimen, 2.05 grams in 12 minutes (0.17 gram per minute).
10.35 a.m.	Intravenous injection of 50 cc. Ringer.
10.45 a.m.	Intravenous injection of 50 cc. Ringer.
10.50 a.m.	Fifth adrenal specimen, collected for 1 minute.
10.51 a.m.	Sixth adrenal specimen, 2.55 grams in 6 minutes (0.425 gram per minute). Another specimen of indifferent blood was now obtained from the right heart. Combined weight of adrenals 0.922 gram.

We have had many illustrations in this work of a fact which may have some general bearing upon the mechanism of spinal shock but whose bearing upon that depression of the spinal mechanism concerned in the liberation of epinephrin sometimes observed after cervical cord transection, and which we suggest is a spinal shock phenomenon, seems incontestable. When the blood pressure and therefore the blood flow through the adrenals are lowered very considerably while the central nervous system is intact, we always find a corresponding increase in the epinephrin concentration in the adrenal vein blood, which until the possible maximum concentration has been attained keeps the output of epinephrin per unit of time as high as the initial output, at any rate for the relatively short duration of the low blood pressure which we have studied. That is to say, with intact nervous system the epinephrin secreting mechanism in the thoracic cord continues to sustain the original output in spite of the impaired circulation. It is, of course, to be supposed that after the low blood pressure has endured for a considerable time (vascular shock) the output will be diminished. But this point is not easily reached in experiments of moderate duration. When, however, injury to the cervical cord is associated with a low blood pressure, which would not of itself cause diminution of the epinephrin output, the output may be markedly diminished and then a slow adrenal blood flow may be accompanied by a great *decline* in the concentration of epinephrin in the adrenal vein blood.

It has often been pointed out that spinal shock cannot be due to the fall of blood pressure since this affects equally the parts of the central nervous system above and below the lesion. No doubt in a general way this is true enough. But if attention be paid to the fact that the direction of flow of the blood in the main spinal arteries is caudad and the direction of flow of a good deal of the venous blood cephalad, it may appear that for some distance below a spinal transection, especially perhaps in the cervical region, the possibility would exist of a greater interference with the circulation below the lesion, for a given fall of blood pressure, than above it, in spite of the lateral anastomoses. In any case, it seems to follow pretty clearly from our observations on cats that the rupture of the upper paths is more likely to cripple the epinephrin secretory mechanism in the thoracic cord if accompanied by an unusually low blood pressure than when the circulation remains relatively efficient after the cervical cord lesion. The loss of impulses from above is the same in both cases, but the well nourished thoracic mechanism goes on sustaining the epinephrin output at a high or at the

original level, whereas the poorly nourished mechanism in the absence of those impulses is unable to do this.

In cats 322, 399, 406 (table 1) the output was diminished after transection of the cord to one-half to one-third of the initial output. When strychnine was given, in two of the animals in which the output had been diminished by the cord section, it was raised not only to the initial value but to three times that amount. Included in table 1 are three experiments in which the upper parts of the central nervous system were eliminated by permanent ligation of the innominate, left subclavian and other arteries supplying the head with blood. There could not be any doubt that at the time of collection of the adrenal blood after ligation of the arteries the elimination of the bulb and everything above it was complete. The eye reflexes had long since disappeared, the respiratory center had ceased to discharge, the swallowing center had become inactive, and so on. The observations of Stewart, Guthrie, Burns and Pike (4) show that after such ligation of arteries in cats there is total elimination of the bulb and everything above it and some interference with upper cervical segments.

In these animals, without exception, the epinephrin output after elimination of the bulb and everything above it by the bloodless method was not at all diminished. It is clear that by this method the infliction of direct injury upon the portion of the cord concerned in sustaining the epinephrin output by such gross interference with its circulation as might be caused by subdural hemorrhage, cutting and blocking of spinal arteries and veins, is largely obviated. The residual blood pressure after elimination of the brain and bulb is probably also in general somewhat higher than when the cervical cord is divided by the knife, and the proportion of experiments in which the output continued without diminution is increased. Two of the protocols follow.

Condensed protocol. Cat 325; male; weight, 2.05 kgm.

9.20 a.m. to 9.50 a.m. Anesthetized with ether, inserted tracheal cannula, placed ligatures around the innominate, left subclavian and both carotid arteries, but did not tie off yet, started artificial respiration (pleura punctured).

10.10 a.m. Cava pocket completed; coeliac axis, renal, mesenteric arteries and abdominal aorta (above bifurcation) tied in addition to the veins entering the cava pocket. Collected adrenal blood.

10.14 a.m. First specimen, 1.2 gram in 30 seconds (2.4 grams per minute).

10.14½ a.m. Second specimen, 5.15 grams in 3 minutes (1.72 gram per minute).

10.22 a.m. Tying off head arteries completed.

10.23 a.m. Pupils maximally dilated. No eye reflexes present. Gums pale. Intra-ocular pressure diminished. No spontaneous respiratory movements.

10.26½ a.m. Third adrenal specimen, 0.6 gram in 30 seconds (1.2 gram per minute).

10.27 a.m. Fourth adrenal specimen, 2.3 grams in 5 minutes (0.5 gram per minute).

10.32 a.m. No change in symptoms showing complete cerebral anemia and no change observed thereafter throughout the rest of the experiment. Obtained (indifferent) venous blood. Combined weight of adrenals, 0.252 gram.

Autopsy. All of the head arteries were tied off (i.e., innominate, left subclavian, proximal to the vertebrals and both carotids).

The 6th specimen was shown to have a higher concentration than the 4th. As the concentration of the 4th specimen was 1: 675,000, the 6th must have had a maximal concentration, although of course with the slow flow a full calculated output could not be expected.

Condensed protocol. Cat 435; female (pregnant); weight 2.5 kgm.

10.15 to 10.45 a.m. Anesthetized with ether, inserted tracheal cannula, placed ligatures around the innominate, left subclavian (proximal to origin of vertebral), and both carotid arteries, but did not tie them off.

11.00 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arreries and abdominal aorta (above bifurcation) tied, in addition to the veins entering the cava pocket. Collected adrenal blood.

11.04 a.m. First specimen, 0.6 gram in 30 seconds (1.2 gram per minute).

11.04½ a.m. Second specimen, 1.9 gram in 2½ minutes (0.76 gram per minute).

11.08 a.m. Cat lightly anesthetized; pupils contracted; eye reflexes good; started tying cerebral vessels.

11.10 a.m. Head arteries tied. Pupils widely dilated and eye reflexes gone. Cat still gasping.

11.12 a.m. Mucosa of mouth and nose pale. Cat still gasps occasionally.

11.14 a.m. Hemostat clamped on left subclavian proximal to ligature.

11.15 a.m. No gasps; all upper reflexes gone, tail and hind leg reflexes still present, notwithstanding ligation of vessels in making cava pocket; intra-ocular tension low; swallowing reflex gone. A nick in nasal septum does not bleed.

11.25 a.m. Third adrenal specimen, 0.8 gram in 30 seconds (1.6 gram per minute).

11.25½ a.m. Fourth adrenal specimen, 2.5 grams in 3 minutes (0.83 gram per minute).

11.38 a.m. All symptoms of complete cerebral anemia still the same.

11.40 a.m. Fifth adrenal specimen, collected for 30 seconds.

11.40½ a.m. Sixth adrenal specimen, 1.65 gram in 6 minutes (0.275 gram per minute). Now tied off the adrenal veins and obtained (indifferent) venous blood from the cava. Combined weight of adrenals 0.314 gram.

Autopsy: Verified the complete ligation of all blood vessels going to the head.

In cat 435 the 2nd adrenal specimen, taken before ligation of the head arteries was somewhat weaker than the 4th specimen, collected 15 minutes after completion of the ligation (fig. 5, confirmed by other observations). Diluted with 3 volumes of indifferent blood it was shown to be somewhat stronger than 1:3,600,000 adrenalin, i.e., the 2nd specimen was somewhat stronger than 1:900,000. It was finally assayed at 1:800,000, corresponding to an output of 0.00095 mgm. per minute for the cat, or 0.00038 mgm. per kgm. per minute.

The 4th specimen was proved to be much stronger than 1:1,350,000, stronger than 1:900,000. Diluted with 3 volumes of indifferent blood

Fig. 5. Intestine tracings. Bloods from cat 435. At *1* and *3* Ringer was replaced by indifferent (venous) blood and this at *2* by the 2nd adrenal specimen (collected before tying off head arteries); at *4* by the 4th adrenal specimen (collected after tying off head arteries). All the bloods were diluted with 3 volumes Ringer. Reduced to one-half.

it was not much different from 1:2,700,000, i.e., the 4th specimen was about the same as 1:675,000. Taking it at 1:700,000 we get 0.0012 mgm. per minute as the output for the cat, or 0.00048 mgm. per kgm. per minute. In other words, the complete elimination of the bulb and brain had left the output fully as great as the initial output.

The 6th adrenal specimen, collected 15 to 20 minutes after the end of collection of the 4th specimen, that is after a period of more than half an hour of complete cerebral anemia, was diluted with 3 volumes of indifferent blood before assay. So diluted it was found to differ little in con-

centration from 1: 1,800,000 adrenalin, being if anything a little stronger i.e., the 6th specimen was equivalent to about 1:450,000 adrenalin. It was decidedly weaker than 1:325,000. Taking it at 1:450,000 we get an output of 0.00061 mgm. per minute for the cat, or 0.00024 mgm. per kgm. per minute. The high concentration shows that at this time the adrenal secretion was still quite efficient in the absence of the bulb and brain. With the slow blood flow it could not of course be expected that a full output could be calculated out from such a concentration.

Two experiments are included in table 1 in which the cervical cord was semisected (cats 383 and 385). In one of these (383) after the semisection the output was reduced to about one-half the initial volume. This cannot be interpreted as indicating that the semisection diminished or abolished the output from the corresponding adrenal, but rather that the cord lesion caused a depression in the output from the two adrenals (spinal shock). For in the other cat (385), division of the right half of the cord did not interfere at all with the output of epinephrin from the right adrenal. In this cat the left adrenal was clipped off during collection of the blood, both before and after the semisection.

In addition to the cats included in table 1 in which segment assays were made of the adrenal blood, the epinephrin output was estimated by the reaction upon the pupil (sensitized by previous removal of the superior cervical ganglion) in two more cats (350 and 353). The condensed protocols follow.

Condensed protocol. Cat 350; male, weight 1.82 kgm.

Left superior cervical ganglion excised 13 days prior to experiment. Left pupil contracted and nictitating membrane forward.

9.00 a.m. Urethane, 4 grams by stomach tube.

10.00 a.m. Left pupil $\frac{2}{3}$, right pupil $\frac{1}{3}$ maximal, both nictitating membranes retracted.

10.00 to 10.20 a.m. Inserted tracheal cannula and exposed cord in mid-cervical region. On exposing cord the left pupil became maximally dilated, the right pupil remained slit-like, both nictitating membranes were retracted. In a few minutes the left pupil came down to $\frac{1}{4}$ maximal dilatation.

10.22 a.m. Started artificial respiration and made "long" pocket, tying the coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.

10.40 a.m. Pocket completed. Right pupil slit-like, left pupil $\frac{1}{8}$ dilated, both nictitating membranes slightly forward, the right more than the left.

10.52 to 11.08 a.m. Two observations showed that a 2-minute pocket was equal to 1.0 cc. of 1: 1,500,000 adrenalin, corresponding to an output of 0.00035 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute.

11.15 a.m. Transected cord through the origins of the 5th pair of cervical nerves (position of cord section was verified at autopsy).

11.18 to 11.26 a.m. Two pocket observations showed a 2-minute pocket to correspond to 1.0 cc. of 1: 1,500,000 adrenal, i.e., the output was the same as before cord section. Combined weight of adrenals, 0.41 gram.

Condensed protocol. Cat 353; old male; weight, 3.13 kgm.

Left superior cervical ganglion excised 19 days prior to experiment. Left pupil contracted and nictitating membrane forward.

9.15 a.m. Urethane, 4 grams by stomach tube.

10.20 a.m. Urethane, 2 grams by stomach tube.

10.45 a.m. Exposed cord in mid-cervical and mid-dorsal regions. Loose ligatures passed under cord.

11.20 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above the bifurcation) were tied in addition to the veins entering the cava pocket.

11.22 a.m. Right pupil slit-like, left pupil ⅓ dilated, both nictitating membranes forward.

11.23 to 11.41 a.m. Three observations showed that a 2-minute pocket was equal to 0.5 cc. of 1: 660,000 adrenalin, corresponding to an output of 0.00037 mgm. per minute for the cat, or 0.00012 mgm. per kgm. per minute.

11.45 a.m. Transected cord just above the origins of the 5th pair of cervical nerves.

11.48 a m. to 12.00 m. Three observations showed that a 2-minute pocket was fully equivalent to 0.5 cc. of 1: 1,000,000 adrenalin, corresponding to an output of fully 0.00025 mgm. per minute for the cat, or 0.00008 mgm. per kgm. per minute. Combined weight of adrenals, 0.705 gram.

ACTION OF STRYCHNINE AFTER CERVICAL CORD TRANSECTION

As already stated, in a number of the cats strychnine was injected after the cervical transection in order to see whether the stimulating action exerted by it upon the epinephrin output in animals with intact cord would be developed after elimination of the upper parts of the central nervous system. This was found to be the case. Increases in the output both relatively and absolutely as great as those seen with intact cord were obtained. In one of the experiments already discussed (cat 407) the initial output remained unchanged after the cord

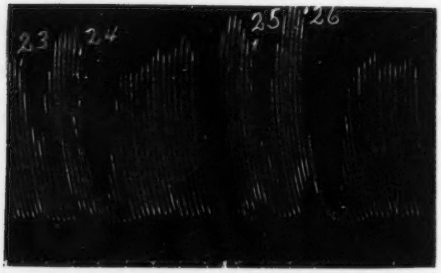

Fig. 6. Intestine tracings. Bloods from cat 406. At *23* and *25* Ringer was replaced by indifferent blood and this at *24* by the 4th adrenal specimen (collected 14 minutes after cervical cord section) and at *26* by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

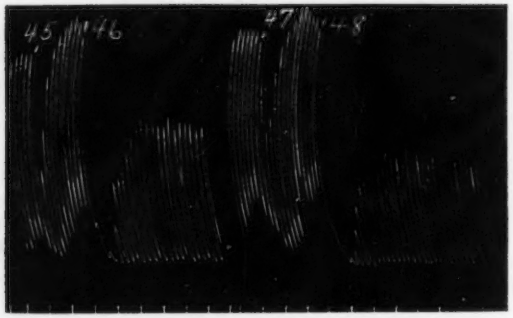

Fig. 7. Intestine tracings. Bloods from cat 406. At *45* and *47* Ringer was replaced by indifferent (venous) blood and this at *46* by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000, and at *48* by the 6th adrenal specimen (collected 5 minutes after intravenous injection of strychnine and 33 minutes after cervical cord section) diluted with 4 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

section and was increased sevenfold by strychnine. In another experiment (cat 406) the output was diminished to something more than half the original output by the cervical transection and was brought up to three times the initial output by strychnine.

Figures 6 to 8 illustrate the assay in cat 406. The 2nd adrenal specimen was found to be much stronger than 1:5,300,000, somewhat stronger than 1:4,000,000 (confirmed by a number of observations), weaker than 1:2,750,000 (confirmed by a number of observations). It was finally taken at 1:3,600,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00024 mgm. per kgm. per minute.

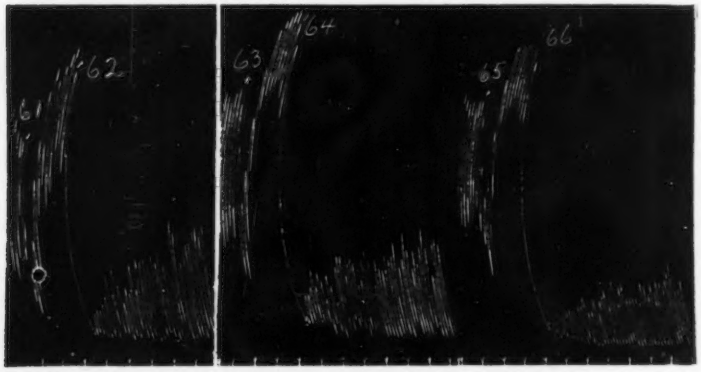

Fig. 8. Intestine tracings. Bloods from cat 406. At *61*, *63* and *65* Ringer was replaced by indifferent (venous) blood and this at *62* by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000; at *64* by the 6th adrenal specimen (collected 5 minutes after intravenous injection of strychnine and 33 minutes after cervical cord section) diluted with 9 volumes of indifferent blood; at *66* by indifferent blood to which was added adrenalin to make a concentration of 1:665,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

The 4th specimen, collected after transection, was weaker than 1:4,000,000 (fig. 6, confirmed by another set of observations), stronger than 1:8,000,000, somewhat stronger than 1:6,600,000. It was taken at 1:6,000,000, corresponding to an output of 0.00026 mgm. per minute for the cat, or 0.00012 mgm. per kgm. per minute.

The 6th specimen, collected after injection of strychnine, had so high a concentration of epinephrin that it was diluted with indifferent blood before assay. Diluted with 4 volumes of indifferent blood it was much stronger than 1:2,750,000, i.e., the 6th specimen was much stronger than 1:550,000. It was similarly found to be stronger than 1:265,000 (fig. 7), and slightly weaker than 1:132,000. In another dilution (with 9 volumes of indifferent blood) it was shown to be stronger than 1:275,000, decidedly weaker than 1:66,000, and not very different from 1:132,000 (fig. 8). The segment was more sensitive at this stage and still greater dilution would have been necessary for the best results. The 6th specimen was finally taken at 1:150,000, corresponding to 0.0013 mgm. per minute for the cat, or 0.00062 mgm. per kgm. per minute.

In a previous paper (5) it was shown that the action of strychnine is a lasting one. This has been illustrated in an interesting fashion in the cord transection experiments, for it has been proved that the excitation of the mechanism on which the strychnine increase in epinephrin output depends, once induced, can survive transection of the cervical cord.

Condensed protocol. Cat 418; female; weight, 1.96 kgm.

9.10 a.m. Anesthetized with ether, inserted cannulae into trachea, carotid artery and external jugular vein. Obtained a specimen of indifferent (jugular) blood.

9.30 a.m. Exposed cord in mid-cervical region. Made cava pocket, tying coeliac axis, renal and superior mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.

10.05 a.m. Cava pocket completed. Collected adrenal blood.

10.07½ a.m. First specimen, 1.05 gram in 30 seconds (2.1 grams per minute).

10.08 a.m. Second specimen, 3.2 grams in 90 seconds (2.1 grams per minute).

10.10 a.m. Blood pressure 105 mm. of mercury.

10.14 a.m. Started artificial respiration (cat breathing well spontaneously).

10.17 a.m. Intravenous injection of 0.25 mgm. strychnine. Clonic spasms occurred within a few seconds and the blood pressure rose to 120 mm. of mercury.

10.21½ a.m. Third adrenal specimen, 0.65 gram in 30 seconds (1.3 gram per minute).

10.22 a.m. Fourth adrenal specimen, 3.35 grams in 2 minutes (1.7 gram per minute).

10.24 a.m. Blood pressure 94 mm. of mercury.

10.30 a.m. Transected cord through origins of 5th pair of cervical nerves (position of cord section verified at autopsy).

10.33 a.m. Blood pressure 24 mm. of mercury. Reflexes exaggerated.
10.34 a.m. Fifth adrenal specimen collected for one minute (not weighed).
10.35 a.m. Sixth adrenal specimen 1.0 gram in 10 minutes (0.1 gram per minute).

Another specimen of indifferent blood was now obtained. Combined weight of adrenals, 0.232, gram.

In figures 9 and 10 are reproduced a small sample of the tracings used in the assay in cat 418.

The 2nd specimen, obtained before injection of strychnine, was found to be stronger than 1:4,500,000 adrenalin, decidedly weaker than 1:3,000,000 and somewhat weaker than 1:3,750,000. It was assayed at 1:4,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute.

The 4th specimen, collected after administration of strychnine, but before transection of the cord, was much stronger than 1:3,000,000, somewhat weaker than 1:1,500,000 (fig. 9, confirmed by other observations). It was taken at 1:1,700,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kgm. per minute, double the initial output.

The 6th specimen, collected after section of the cord, had a very high concentration of epinephrin. It was diluted with 3 volumes of indifferent blood before assay. Thus diluted it was stronger than 1:750,000 adrenalin, i.e., the 6th specimen was stronger than 1:185,000 (fig. 10, confirmed by other observations). It was probably somewhat weaker than 1:94,000 and was taken at 1:100,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kgm. per minute.

Since the output, increased by strychnine, was not changed by the cervical transection it follows that in this animal the influence of strychnine in increasing the epinephrin output was, within the limits of error of the assay, exerted exclusively on the portion of the cord below the transection. It is of interest that in spite of the low blood pressure which followed the transection (to 24 mm. of mercury), spinal shock of the epinephrin secretory mechanism in the thoracic cord, as manifested by a fall in the rate of output, did not occur, the marked diminution in the rate of blood flow through the adrenals being compensated for by the great increase in epinephrin concentration. Where the mechanism has suffered from the condition which we suggest is analogous to spinal shock affecting other centers, a reduced blood flow may be accompanied by a diminished concentration of epinephrin, a combination practically never encountered under the conditions of our

experiments while the central nervous system is intact. The administration of strychnine in this experiment might be said to have constituted a prophylaxis against spinal shock so far as the epinephrin secretory mechanism is concerned.

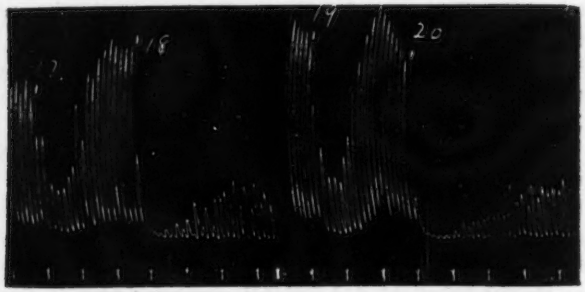

Fig. 9. Intestine tracings. Bloods from cat 418. At *17* and *19* Ringer was replaced by indifferent (venous) blood and this at *18* by the 4th adrenal specimen (collected 5 minutes after intravenous injection of strychnine); at *20* by indifferent blood to which was added adrenalin to make a concentration of 1: 1,500,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

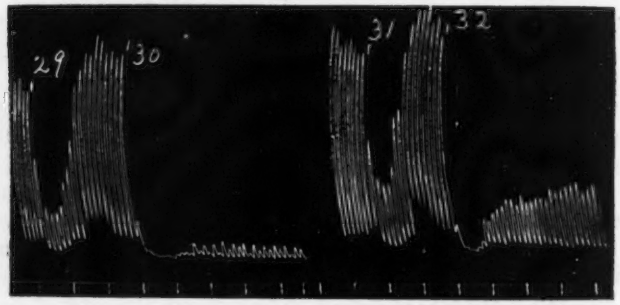

Fig. 10. Intestine tracings. Bloods from cat 418. At *29* and *31* Ringer was replaced by indifferent (venous) blood and this at *30* by the 6th adrenal specimen (collected 18 minutes after intravenous injection of strychnine and 5 minutes after cervical cord section) diluted with 3 volumes of indifferent blood; at *32* by indifferent blood to which was added adrenalin to make a concentration of 1: 750-000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

In contrast to the action of strychnine on animals with intact central nervous system (5) in which the maximum concentration of epinephrin was not found to surpass substantially the maximum observed without strychnine, the maximum concentration under the influence of strychnine in the cats with cervical cord transection was observed to reach extraordinarily high values (1:70,000 in cat 407; 1:100,000 in cat 418; 1:150,000 in cat 406; 1:250,000 in cat 399). This is consistent with the suggestion expressed in a previous paper (5) that the preliminary diminution in the output produced by strychnine in animals with intact central nervous system may be due to transient stimulation by the drug of a regulatory (inhibitory) mechanism situated higher up and cut off by the cervical transection.

The 2nd specimen from cat 399 (before transection) was shown on intestine segments to be decidedly stronger than 1:7,500,000, stronger than 1:6,000,000, somewhat weaker than 1:4,500,000. It was taken at 1:5,000,000, corresponding to an output of 0.00065 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute.

The 4th specimen (after transection) was much stronger than the 2nd. Diluted with 2 volumes of indifferent blood it was still stronger than 1:6,000,000, i.e., the undiluted 4th specimen was stronger than 1:2,000,000. It was found to be decidedly weaker than 1:750,000, weaker than 1:1,000,000 and not far different from 1:1,500,000, corresponding to an output of 0.0002 mgm. per minute, for the cat, or 0.000075 mgm. per kgm. per minute. Considering the small flow this is a fair output, but the striking thing is that the concentration, although increased to more than three times that of the 2nd specimen, corresponding to the diminished blood flow, is not lifted nearly to the possible maximum as would occur with intact cord, whereas when strychnine is given the concentration is increased sixfold, to much beyond the maximum seen without strychnine, while the blood flow is also increasing somewhat.

The assay on intestine segments showed that the 6th specimen (from cat 399) was much stronger than the 4th, much stronger than 1:750,000, much stronger than 1:535,000, much stronger than 1:415,000, stronger than 1:320,000, decidedly weaker than 1:180,000 and not far different from 1:250,000 adrenalin. In making these tests the adrenal blood was diluted with 2 volumes and in other observations with 4 volumes of indifferent blood. It was confirmed on the uterus (fig. 11) that the 6th specimen was much stronger than the others.

That the strychnine action is essentially a central action is best shown by administering the drug to animals from which one adrenal has been previously removed and the nerves of the other cut. Where the output has been reduced below the point of detection or, if detectable, to a small fraction of the normal average, it does not become detectable, or is not increased on injecting strychnine. On the other hand, if a substantial, although of course much reduced output, is present after the adrenal operation, either because the nerves have been incompletely divided or some regeneration has occurred after a long survival period, strychnine causes a definite increase. Illustrations of these facts have already been published in a previous paper (6).

The same thing may be proved by sectioning the adrenal nerves in acute experiments which, however, are on the whole less satisfactory on account of the fall of blood pressure produced by the denervation. A protocol from one of these experiments (cat 431) is given as an example.

Condensed protocol. Cat 431; female; weight, 2.05 kgm.

9.20 a.m. Anesthetized with ether; inserted tracheal and jugular cannulae; obtained indifferent (jugular) blood.

9.55 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied in addition to the veins entering the cava pocket. Collected adrenal blood.

9.55½ a.m. First specimen, 1.95 gram in 30 seconds (3.9 grams per minute).

9.56 a.m. Second specimen, 7.0 grams in 2 minutes (2.5 grams per minute).

Fig. 11. Uterus tracings. Bloods from cat 399. At *62* Ringer was replaced by the 6th adrenal blood specimen (collected 3 minutes after intravenous injection of strychnine and 23 minutes after cervical cord section) diluted with 6 volumes of indifferent blood; at *63* by indifferent (venous) blood; at *64* by indifferent blood to which was added adrenalin to make a concentration of 1: 2,250,000; at *65* by the 6th specimen diluted with 4 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). At *66* Ringer was replaced by the 6th specimen; at *68* by the 4th specimen (collected 3½ minutes after cervical cord section); at *69* by the 2nd specimen (collected before cord section). The bloods 66–69 were diluted with 10 volumes Ringer. Reduced to one-half.

10.00 a.m. Started artificial respiration (cat still breathing well spontaneously).
10.07 a.m. Intravenous injection of 0.35 mgm. strychnine (sulphate, as in all the experiments).
10.07½ a.m. Clonic convulsions; spontaneous breathing good.
10 22 a.m. Clonic spasms; spontaneous breathing good.
10·23 a.m. Third adrenal specimen, 1.05 gram in 1 minute.
10 24 a.m. Fourth adrenal specimen, 3.85 grams in 3 minutes (1.28 gram per minute).
10.30 to 10.32 a.m. Cut right and left major and minor splanchnics (in abdomen) and extirpated the left semilunar ganglion.
10.34 a.m. Clonic spasms at intervals.
10.38 a.m. Fifth adrenal specimen, collected for 30 seconds.
10.38½ a.m. Sixth adrenal specimen, 2.7 grams in 10 minutes (0.27 gram per minute).

Another specimen of indifferent (venous) blood was now obtained. Combined weight of adrenals, 0.25 gram. Section of nerves verified at autopsy.

Figures 12 to 15 give samples of the tracings used in the assay in cat 431.

The 2nd adrenal specimen was found to be stronger than 1:8,300,000, weaker than 1:5,000,000, weaker than 1:6,600,000 adrenalin. It was assayed at 1:7,500,000, corresponding to an output of 0.00045 mgm. per minute for the cat, or 0.00023 mgm. per kgm. per minute.

The 4th specimen, obtained after administration of strychnine, was decidedly stronger than 1:1,660,000, stronger than 1:830,000. It was much stronger than the 6th specimen, collected after section of nerves to the adrenals in spite of the greatly diminished flow for the 6th specimen. This was evident when both specimens were diluted with 3 volumes of Ringer's solution, but became still more apparent on first diluting each with 3 volumes of indifferent blood and then diluting the mixtures with 3 volumes of Ringer (fig. 12). The 4th specimen diluted with 3 volumes of indifferent blood was weaker than 1:1,660,000 adrenalin, i.e., the 4th specimen was weaker than 1:415,000 (fig. 13). The 4th specimen was finally assayed at 1:700,000, corresponding to an output of 0.0018 mgm. per minute for the cat, or 0.0009 mgm. per kgm. per minute, four times the initial output.

The 6th specimen, diluted with 1 volume of indifferent blood, was weaker than 1:3,300,000, i.e., the 6th specimen was weaker than 1:1,650,000. Undiluted with indifferent blood it was found to be stronger than 1:3,300,000, but decidedly weaker than 1:1,660,000 (fig. 14). It was assayed at 1:2,000,000, corresponding to an output of 0.00013 mgm. per minute for the cat, or 0.00006 mgm, per kgm. per minute, only one-fifteenth of the output under strychnine before section of the nerves.

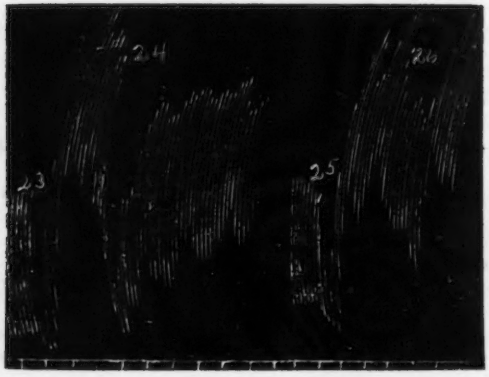

Fig. 12. Intestine tracings. Bloods from cat 431. At *23* and *25* Ringer was replaced by indifferent (venous) blood and this at *24* by the 4th adrenal specimen (collected 17 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at *26* by the 6th adrenal specimen (collected after section of nerves to both adrenals) diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer. Reduced to one-half.

Fig. 13. Intestine tracings. Bloods from cat 431. At *35* and *37* Ringer was replaced by indifferent (venous) blood and this at *36* by the 4th adrenal specimen (collected 17 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at *38* by indifferent blood to which was added adrenalin to make a concentration of 1: 1,660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

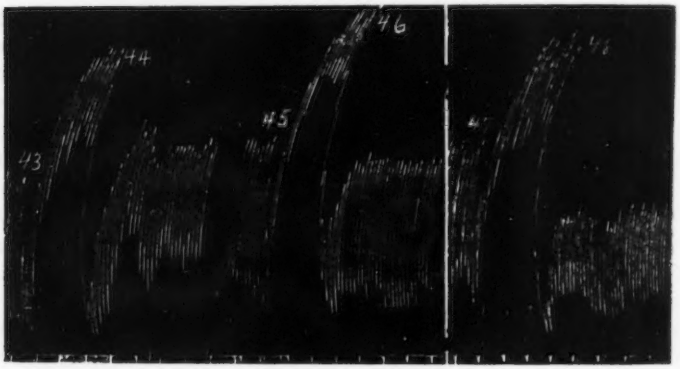

Fig. 14. Intestine tracings. Bloods from cat 431. At *43*, *45* and *47* Ringer was replaced by indifferent (venous) blood and this at *44* by indifferent blood to which was added adrenalin to make a concentration of 1: 3,300,000; at *46* by the 6th adrenal specimen (collected after section of nerves to both adrenals); at *48* by indifferent blood to which was added adrenalin to make a concentration of 1: 1,660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

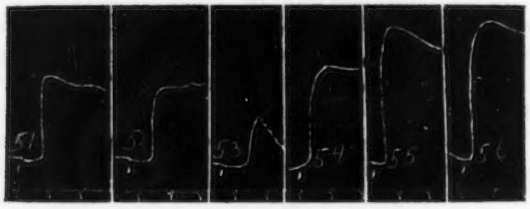

Fig. 15. Uterus tracings. Bloods from cat 431. At *51* Ringer was replaced by the 4th adrenal specimen (collected after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at *52* by the 6th adrenal specimen (collected after section of nerves to both adrenals) diluted with 1 volume of indifferent blood; at *53* by indifferent (venous) blood; at *54* by the 2nd adrenal specimen (collected before injection of strychnine); at *55* by the 4th adrenal specimen; at *56* by the 4th adrenal specimen diluted with 1 volume of indifferent blood. All the bloods were diluted with 3 volumes Ringer. Reduced to two-thirds.

It was confirmed by the uterus that the 4th specimen, diluted with 3 volumes of indifferent blood, was stronger than the 2nd specimen undiluted with indifferent blood, and at any rate not weaker than the 6th specimen diluted with 1 volume of indifferent blood. The 4th specimen was clearly much stronger than the 2nd (fig. 15).

This experiment is a complete contrast to that on cat 318 in which the cervical cord was transected after the administration of strychnine. There the output of epinephrin, increased by the strychnine, went on unchanged. In the present experiment strychnine markedly increased the output, but even incomplete section of the peripheral adrenal secretory paths caused a great diminution.

ACUTE EXPERIMENTS ON OTHER ANIMALS THAN CATS

Table 2 summarizes the results of acute experiments on cervical section in 3 dogs, 2 rhesus monkeys and 1 rabbit. Two experiments on dogs in which the cord was semisected in the cervical region are included in the table. In the dogs and monkeys the output after cervical cord transection was always less than before the transection, except in one of the monkeys where the initial output was abnormally low (shock after exposure of the cord). In this case, as noted in cats under similar conditions, transection of the cord caused no further diminution in the epinephrin output. Semisection of the cervical cord in dogs yielded results of the same general character as in cats. In one of the experiments in the table there was no change in the output after the semisection, in the other it was reduced.

In addition to the acute experiments on dogs summarized in table 2, a number of experiments with blood pressure auto-assays were made. For example, in a dog (351) weighing 6.3 kgm. the output was estimated at 0.00015 mgm. per kgm. per minute. The blood pressure was 94 mm. of mercury. The cord was then transected through the 5th cervical pair. The blood pressure fell to 55 mm. of mercury. The output of epinephrin was now estimated at 0.00003 mgm. per kgm. per minute. At the end of the experiment a sample of adrenal blood, collected at the rate of 1.0 gram per minute assayed (on rabbit segments) at 1:3,000,000 corresponding to an output of 0.00005 mgm. per kgm. per minute.

SURVIVAL EXPERIMENTS

In table 3 are displayed the results of experiments on 6 cats and 4 dogs which were allowed to survive transection of the cervical cord for 2 to 13 days. It will be seen that while in most of the animals an output of epinephrin measurable by segments of the sensitiveness employed was present, the output never attained the normal average in animals with intact cord. In the dogs the average output after cervical cord transection is considerably greater than in the cats, the opposite of what is found in the acute experiments (see tables 1 and 2). The suggestion is that the mechanism in the thoracic cord is more easily depressed by the cervical transection (spinal shock), but recovers more easily in survival experiments in the dog than in the cat. We do not know whether this apparently easier recovery means anything more than that the dogs in general in our survival experiments did withstand cervical transection better than the cats. They ate better, passed urine and feces more normally and lived longer. Most of the cats during the time they lived or were permitted to live neither micturated nor defecated, although the bladder was generally much distended with urine. In several instances the bladder was artificially emptied in the cats. This was not necessary in the dogs. It is well known that after these high cord transections one of the most important precautions to be taken is to keep up the body temperature. Although all the animals were kept in a room specially warmed day and night, the dogs were somewhat larger than the cats and therefore it is to be supposed would not cool so easily, but we do not know whether this was a factor in their better condition. Most of the animals were in fair, some (including those taken after the longest periods) in very good condition when sacrificed. It would be desirable to make observations on animals after much longer periods of survival.

While it is clear from these experiments, as from the previous ones, that a substantial liberation of epinephrin, sustained from the thoracic cord, may be present in animals which have survived cervical cord transection 2 to 13 days, it is impossible to say whether with a longer survival period the output would approach more nearly to the normal average or not. Great quantitative and possibly some qualitative changes in metabolism follow such a lesion, and in the present state of our knowledge it would be useless to speculate as to how these might affect the upbuilding or output of epinephrin. Mere inanition for 3 days, and all these animals, of course, take little food for some time

TABLE 2

Acute experiments. Dogs, etc.

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD							REMARKS
	Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		
									For animal	Per kilogram						For animal	Per kilogram	
			gms.	*gms.*	*sec.*	*gms.*	*mm. of mercury*		*mgm.*	*mgm.*	*gms.*	*sec.*	*gms.*	*mm. of mercury*		*mgm.*	*mgm.*	
327	7.4	v–vi	0.615	12.35	30	24.7	130	1:15,000,000	0.0016	0.0002	9.5	60	9.5	38	1:40,000,000*		0.00003*	
											10.0	120	5.0	36	1:40,000,000*			
352	6.5	below vi	1.886	9.4	120	4.7	98	1:3,300,000	0.0014	0.0002	6.4	120	3.2	72	1:5,000,000	0.00064	0.0001	After cord section blood pressure fell to 18 mm. of mercury; 300 cc. Ringer injected at once and blood pressure rose to 72 mm. of mercury
380	5.5	v–vi	1.082	9.7	60	9.7	120	1:4,500,000	0.002	0.00035	2.25	240	(a)0.56	40	1:1,900,000	0.0003	0.000055	Ringer's solution injected between collection of (*a*) and (*b*). 100 cc.
											4.1	120	(b)2.05	52	1:8,500,000	0.00025	0.000045	

328	1.87	vii	0.412	4.5	180	1.5	58	1:9,000,000	0.00017	0.0001	4.45	120	2.22	62	1:13,000,000	0.00017	0.0001	50 cc. Ringer injected before and 50 cc. after cord section
250	8.8	vi	0.796	9.2	150	3.7		1:8,000,000	0.00045	0.00005	10.25	120	5.12		1:13,000,000	0.0004	0.000045	Ringer injected after cord section. 100 cc.
276	8.4	below viii	0.947	6.1	60	6.1		1:5,000,000	0.0012	0.00014	5.05	150	2.0		1:9,000,000	0.00022	0.000026	Assay of specimen, taken after cord section, completed next day
379	4.8	above vi	0.894	8.15	90	5.4	140	1:3,000,000	0.0018	0.00037	7.5	180	2.5	97	1:1,400,000	0.0018	0.00037	Both adrenals discharging
382	7.2	iv-v	0.870	10.95	90	(a)7.3	120	1:4,000,000	0.0018	0.00025	5.1	120	(b)2.6	90	1:5,000,000	0.0005	0.00007	(*a*)before semisection; (*b*) after semisection; (*c*) after transection below v. Both adrenals discharging
											4.2	180	(c)1.4	44	1:2,800,000	0.0005	0.00007	

Note. 379 and 382 were semisections (right), the rest transections. 328 was a rabbit, 250 and 276 monkeys, the rest dogs.

* These are the maximum concentrations and output which could have been present. The segment could have detected these concentrations had they been present in the adrenal bloods.

after the operation, does not appear, so far as can be judged from one experiment, to have any notable influence. Thus a cat weighing 2.22 kgm. received no food for 3 days. The stomach and intestines were empty except for some feces in the lower colon. Under urethane specimens of adrenal blood were obtained in the usual way. The 2nd specimen (1.67 gram per minute) was assayed at 1:4,000,000 epinephrin, corresponding to an output of 0.00042 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute. The 4th specimen (1.27 gram per minute) was assayed at 1:2,800,000 epinephrin, corresponding to 0.00045 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute.

It was shown that strychnine exerted its stimulating effect upon the epinephrin output in these survival experiments, doubtless by acting upon the thoracic cord mechanism, which was already sustaining whatever output was going on. Where the initial output is quite small, as in a cat (424) in which the transection was made not in the cervical region but between the first and second dorsal segments, the relative increase produced by strychnine may be very great.

Condensed protocol. Cat 424; female; weight, 2.1 kgm.

December 9. Excised left superior cervical ganglion.

December 20. Cord transected between origins of 1st and 2nd dorsal nerves. Immediately after cord section (while still anesthetized), the left pupil became wider than the right (both dilated), but on recovery the left pupil contracted and left nictitating membrane came forward as in animals without cord section.

December 24. Condition good. Left pupil contracted and nictitating membrane forward.

10.10 a.m. Administered a little ether to insert tracheal and jugular cannulae and obtained indifferent (jugular) blood. Isolated right and left vago-sympathetic nerves on loose ligatures. Thereafter no more ether was needed.

10.30 a.m. Left pupil contracted and nictitating membrane forward. Cut left, then right vago-sympathetic nerves. After section of the right vago-sympathetic both pupils became smaller but the left remained smaller than the right; left nictitating forward.

10.45 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied, in addition to the veins entering the cava pocket. The operative field was of course totally insensitive owing to the cord section.

10.50 a.m. Left pupil wider than right; both nictitating membranes forward.

10.52 a.m. Left pupil wider than right; left nictitating back; left aperture wider than right.

10.55 a.m. to 11.20 a.m. Two 2-minute and one 3-minute pocket observations gave no reactions with the pupil; 0.5 cc. 1:650,000 adrenalin gave a good pupil reaction. Collected adrenal blood.

11.30 a.m. First specimen, 0.45 gram in 30 seconds (0.9 gram per minute).

11.30½ a.m. Second specimen, 5.05 grams in 6 minutes (0.84 gram per minute).

11.42 a.m. Started artificial respiration.

11.45 a.m. Intravenous injection of 0.5 mgm. strychnine. In a few seconds a tonic convulsion occurred which was followed after a few seconds by clonic spasms. During the spasms the left pupil became maximally dilated, the right pupil about one half dilated, the left nictitating membrane retracted and the right forward. On clipping off the pocket for 3 minutes the left pupil came down somewhat and the nictitating membrane came forward slightly, but the left pupil remained wider than the right.

11.51½ a.m. Third adrenal specimen, 0.5 gram in 30 seconds (1.0 gram per minute).

11.52 a.m. Fourth adrenal specimen, 3.7 grams in 7 minutes (0.53 gram per minute). On release of the pocket clip after collecting the 4th specimen, a good pupil and nictitating reaction was observed on the left side although the left pupil was already wide). Another specimen of indifferent blood was now obtained.

The 2nd specimen (taken before strychnine injection) was assayed at 1:150,000,000. This corresponds to an output of only 0.0000055 mgm. per minute for the cat, or 0.0000025 mgm. per kgm. per minute, one-hundredth of the normal average. Figure 16 gives some of the tracings used in the assay of the 2nd specimen. It was weaker than 1:130,000,000 adrenalin, stronger than 1:195,000,000. Other tracings, not reproduced, showed that it was much weaker than 1:65,000,000. Fortunately the segment was exceptionally sensitive, as will be seen from the excellent reactions given with blood so poor in epinephrin. It must be remembered that, as applied to the segment, the bloods being diluted with 3 volumes of Ringer's solution, the actual concentrations of epinephrin were only one-fourth of those given. This illustrates a point of technique on which we have often insisted, but which is still overlooked by some investigators, namely, that the mere occurrence of a large inhibitory reaction of an intestine segment gives no information as to the concentration of epinephrin in the blood producing it until the sensitiveness of the segment for epinephrin has been quantitatively determined.

It was shown that the 4th specimen, collected after strychnine, was much stronger than 1:6,500,000, and stronger than 1:1,300,000 adrenalin. Diluted with 3 volumes of indifferent blood it was not much different from 1:3,900,000, i.e., the 4th specimen was not far from

TABLE 3

Cervical cord sections. Survival experiments

NUMBER OF ANIMAL	BODY WEIGHT	LEVEL OF CORD SECTION	PERIOD OF SURVIVAL	COMBINED WEIGHT OF ADRENALS	BLOOD COLLECTED	DURATION OF COLLECTION	BLOOD FLOW PER MINUTE	EPINEPHRIN CONCENTRATION	EPINEPHRIN OUTPUT PER MINUTE		REMARKS
									For animal	Per kilogram	
Cats			*Days*	*gms.*	*gms.*	*sec.*	*gms.*		*mgm.*	*mgm.*	
332	2.02	vii	2	0.318	3.95	240	1.0	1:30,000,000*	0.00003*	0.000015*	
336	2.53	vii	3	0.383	4.2	720	0.35	1:3,500,000	0.0001	0.00004	
337	2.6	above vi	3	0.535	3.85	180	1.3	1:30,000,000*	0.00004*	0.000015*	Ether administered between collection of these specimens
					2.75	300	0.55	1:30,000,000*	0.000015*	0.000006*	
346	1.72	vi	7	0.378	4.55	300	0.91	1:25,000,000	0.000036	0.00002	Blood pressure at beginning of experiment was 80 mm. of mercury
					2.35	420	0.34	1:10,000,000	0.000034	0.00002	Ether administered between collection of these specimens
420	1.6	below vi	3	0.290	3.35	600	0.335	1:125,000,000	0.0000025	0.0000015	
422	2.4	below viii	2	0.346	2.55	180	0.85	1:25,000,000	0.000035	0.000015	Pupil assay showed output was not more than 0.000025 mgm. per kgm. per minute
419	1.57	below vii	20 hrs.	0.461	3.95	240	1.0	1:30,000,000	0.000033	0.00002	

Dogs											
330	4.0	vii	2	0.810	4.2	600	0.42	1:10,000,000	0.00004	0.00001	Very small blood flow. Circulation feeble. Temperature low
345	3.95	vii–viii	8	0.930	8.55	150	3.4	1:12,000,000	0.00028	0.00007	Ether administered between collection of these specimens
					5.0	180	1.7	1:8,500,000	0.0002	0.00005	
347	2.93	vii–viii	13	1.008	9.7	120	4.8	1:40,000,000	0.00012	0.00004	0.5 mgm. strychnine administered between collection of these specimens
					5.35	240	1.34	1:6,600,000	0.0002	0.00007	
395	4.5	v–vi	11	0.938	5.8	90	3.9	1:60,000,000	0.000065	0.000015	1st specimen collected without etherization. Blood pressure 94 mm. of mercury
					4.25	90	2.8	1:60,000,000	0.000045	0.00001	2nd specimen collected after etherization. Blood pressure 76 mm. of mercury
386†	5.25	below v	8	0.831	11.25	120	(a)5.6	1:8,500,000	0.00066	0.00013	(a) Both adrenals discharging
					4.85	120	(b)2.42	1:6,500,000	0.00035	0.000065	(b) Right adrenal only discharging
					4.85	120	(c)2.42	1:6,500,000	0.00035	0.000065	(c) Left adrenal only discharging

* These are maximum outputs and concentrations which could have been present. The segments, which were not particularly sensitive, could have detected these concentrations had they been present in the adrenal blood.

† 386 is a hemisection experiment (left); the others are transections.

1: 1,000,000. It was decidedly stronger than 1: 1,625,000 (fig. 17, observations 34 and 36), weaker than 1: 650,000 (fig. 17, observations 38 and 40). Another series of observations was made in which the 4th specimen was diluted with 7 volumes and with 14 volumes of indifferent blood, in order to reduce the inhibitory reactions so as to permit of a more exact assay. In this way also it was shown that the specimen was stronger than 1: 1,300,000 and weaker than 1: 870,000. It was finally taken at 1: 1,000,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute, the normal average, and not less than one hundred times the output before strychnine. It was confirmed by the uterus that the

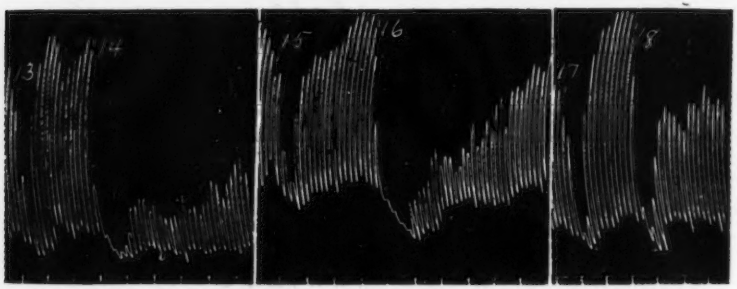

Fig. 16. Intestine tracings. Bloods from cat 424. At *13*, *15* and *17* Ringer was replaced by indifferent (venous) blood and this at *14* by indifferent blood to which was added adrenalin to make a concentration of 1: 130,000,000; at *16* by the 2nd adrenal specimen (collected before injection of strychnine); at *18* by indifferent blood to which was added adrenalin to make a concentration of 1: 195,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

4th specimen gave a good epinephrin reaction, whereas the 2nd specimen and indifferent blood in the same dilution gave no reaction. The uterus was not particularly sensitive.

Advantage was taken of the fact that it was possible to obtain adrenal vein blood without the administration of an anesthetic to test the question whether ether causes any measurable increase in the output after transection of the cervical cord. In no case was any such effect produced. With intact central nervous system we have previously found that the average output is if anything somewhat less with ether than with urethane (6). An observer who trusts to the (denervated)

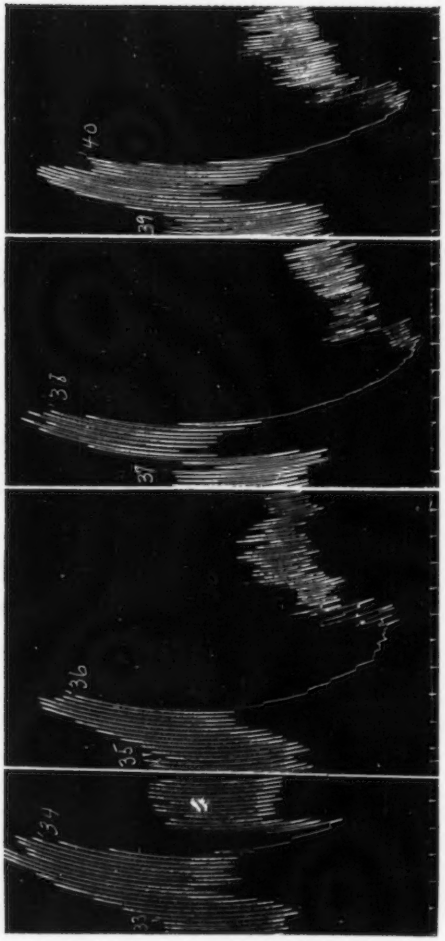

Fig. 17. Intestine tracings. Bloods from cat 424. At *33*, *35*, *37* and *39* Ringer was replaced by indifferent (arterial) blood and this at *34* by indifferent blood to which was added adrenalin to make a concentration of 1:6,600,000; at *36* by the 4th adrenal specimen (collected 8 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at *38* by indifferent blood to which was added adrenalin to make a concentration of 1:2,700,000; at *40* by the 4th adrenal specimen diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

eye reactions alone can easily be deceived by the fact that ether increases the sensitivity of the test object to epinephrin. For instance (in cat 336), a 2-minute pocket without anesthetic gave no reaction. Ether was then administered and now a 2-minute pocket gave an excellent pupil reaction. This was confirmed several times. It was shown that the increased reaction could not be interpreted as due to an augmentation of the epinephrin output by etherization since the reaction to one and the same dose of adrenalin artificially injected was also increased by giving ether. This is illustrated in the following experiment.

Condensed protocol. Cat 423; female; weight, 1.8 kgm.

December 9. Excised left superior cervical ganglion.

December 20. Left pupil contracted and nicitating membrane forward. Transected cord below origin of 8th pair of cervical nerves. Immediately after the cord section the left pupil was wider than the right and the right nictitating protruded more than the left.

December 23. Condition good. Left pupil wider than right as it had been since the cord section, both nictitating membranes slightly forward, apertures of both eyes about the same.

9.45 a.m. Administered a little ether to insert tracheal and jugular cannulae. On etherizing, the left pupil became almost maximal, the right pupil dilated but not so widely as the left, the right nictitating protruded more than the left.

10.15 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied in addition to the veins entering the cava pocket.

10.16 a.m. Left pupil $\frac{1}{2}$ dilated; right pupil slit-like; left aperture slightly wider than right.

10.18 a.m. Pocket observation 2 minutes; no eye reaction.

10.22 a.m. Pocket observation 3 minutes; no eye reaction.

10.25 to 10.35 a.m. 0.5 cc. adrenalin 1:2,700,000, and 0.5 cc. adrenalin 1:2,000,000 gave no reaction; 0.5 cc. adrenalin 1:1,330,000 gave a pupil reaction in 11 seconds.

10.35 a.m. Pocket observation 4 minutes, doubtful reaction if any.

10.40 a.m. Etherized to surgical anesthesia.

10.45 a.m. Pocket observation 3 minutes gave a pupil reaction in 14 seconds and retraction of nictitating membrane 2 seconds later.

10.50 a.m. 0.5 cc. adrenalin 1:2,000,000 gave a very good pupil reaction in 11 seconds.

10.55 a.m. 0.5 cc. adrenalin 1:2,700,000 gave a definite reaction in 8.6 seconds; 0.5 cc. adrenalin 1:4,000,000, doubtful if any reaction.

This experiment brings out another point, namely, the reversal of the eye phenomena after section of the cord, which Schafer (7) has called attention to as occurring after section of the cervical sympathetic

on the side opposite to that on which the superior cervical ganglion has been excised. The same reversal was observed on section of the cord just below the 8th cervical segment in cat 422. The left superior cervical ganglion (in cat 422) was excised a fortnight before section of the cord. Up to the time when the cord was divided the left pupil was smaller than the right and the left nictitating membrane was less retracted than the right. On section of the cord the left pupil became wider than the right and the right nictitating protruded further than the left. This condition persisted for the two days during which the animal was allowed to survive. At the end of the experiment in which adrenal blood was obtained, the observation was made when the animal was being bled to death that with the onset of asphyxia the left pupil dilated widely but not so widely as the right which went to maximal dilatation. The right nictitating became completely retracted, while the left was still visible. The right aperture became wider than the left and remained so until death. No anesthetic was administered as of course the whole operative field was completely insensitive. In cat 424, in which the cord was transected between the first and second dorsal segments, the reversal of the eye phenomena was not seen.

OBSERVATIONS ON THE ADRENAL EPINEPHRIN STORE AFTER TRANSECTIONS OF THE CORD

In many of the animals the epinephrin store of the adrenals was estimated at the end of the experiment. The results are collected in table 4. In the two acute experiments, one on a monkey, the other on a dog, the load is less than would be expected in a normal animal killed without anesthetic. But these animals were necessarily anesthetized for some time before the cord transection, and thereafter, nothing can be deduced from this as to any influence of the cord lesion on the store. In the survival experiments, in many of which little or no anesthetic was required for the final experiment, the field of operation being insensitive, there is no conspicuous difference in the store from what would be expected in normal animals. If it looks somewhat too low in some of the animals, it is fully as great as the normal load in others. The effect of post-operative depletion, following the initial operation in which the cord was divided cannot be altogether excluded, although it is not evident. For example, in cat 419, which was taken 20 hours after the cord section, there was a full load.

TABLE 4

NUMBER OF ANIMAL	KIND OF ANIMAL	WEIGHT OF ADRENALS		EPINEPHRIN CONTENT		REMARKS
		Left	Right	Left	Right	
		grams	*grams*	*mgm.*	*mgm.*	
333	Cat	0.231	0.230	0.16	0.16	Cervical cord section below 6th segment: survival 3 days
335	Cat	0.151	0.155	0.16	0.16	Cervical cord section above 7th segment: survival 3 days
336	Cat	0.197	0.186	0.14	0.14	Cervical cord section through 7th segment: survival 3 days
337	Cat	0.257	0.278	0.16	0.17	Cervical cord section above 6th segment: survival 3 days
346	Cat	0.192	0.186	0.23	0.23	Cervical cord section through 6th segment: survival 7 days
348	Cat	0.228	0.224	0.26	0.26	Dorsal cord section below 5th segment: survival 6 days
419	Cat	0.241	0.220	0.26	0.26	Cervical cord section below 7th segment: survival 20 hours
420	Cat	0.152	0.148	0.15	0.15	Cervical cord section below 6th segment: survival 3 days
421	Cat	0.230	0.218	0.22	0.22	Cervical cord section through 7th segment: survival 4 days
422	Cat	0.184	0.162	0.13	0.14	Cervical cord section below 8th segment: survival 2 days
423	Cat	0.208	0.200	0.19	0.19	Cervical cord section below 8th segment: survival 3 days
338	Dog	0.397	0.372	0.42	0.40	Cervical cord section below 7th segment; survival 5 days
345	Dog	0.480	0.450	0.46	0.47	Cervical cord section below 7th segment: survival 8 days
347	Dog	0.488	0.520	0.64	0.66	Cervical cord section below 7th segment: survival 13 days
349	Dog	0.722	0.708	1.31	1.33	Dorsal cord section below 5th segment: survival 7 days
352	Dog	0.964	0.922	0.41	0.42	Acute experiment: morphine and ether: cervical cord section below 6th segment: also dorsal section
394	Dog	0.406	0.352	0.47	0.46	Cervical cord section below 5th segment: survival 8 days
395	Dog	0.520	0.418	0.48	0.48	Cervical cord section below 5th segment: survival 11 days
276	Monkey	0.480	0.467	0.20	0.20	Urethane anesthesia: acute experiment: cord section below last cervical segment: adrenals assayed next day

TABLE 5

NUMBER OF ANIMAL	WEIGHT OF ADRENALS		EPINEPHRIN CONTENT		REMARKS
	Left	Right	Left	Right	
	grams	*grams*	*mgm.*	*mgm.*	
339	0.212	0.186	0.26	0.20	Under ether cervical cord sectioned below 7th segment, left adrenal excised and ether administered for 3 hours and 15 minutes, then removed right adrenal
340	0.160	0.148	0.16	0.16	Cat died 8 days after denervation of left adrenal and 2 days after cervical cord section between 5th and 6th segments
341	0.330	0.300	0.34	0.34	Left adrenal denervated 9 days; cervical cord transected below 6th segment 3 days; cat dying when adrenals were removed
342	0.344	0.320	0.31	0.31	Left adrenal denervated 9 days; cervical cord transected below 6th segment 3 days; ether 2 hours; cat in moribund condition
343	0.210	0.236	0.25	0.16	Under ether sectioned cervical cord (between 5th and 6th segments); excised left adrenal; then morphine 60 mgm., injected hypodermically; removed right adrenal 5½ hours later
344	0.22	0.24	0.21	0.21	Under ether, sectioned cervical cord (above 6th segment); excised left adrenal; then 6 cc. of 2 per cent solution of β-tetrahydronapthylamine injected hypodermically; right adrenal excised 5 hours later

In table 5 are given the results on 6 cats in which a differential effect on the two adrenals could have been manifested, either because one gland had been previously denervated or because one adrenal was removed early and the other at the end of the experiment. In the one experiment tried, morphine seemed to produce its ordinary differential depletion after cervical transection, qualitatively at any rate. It would be impossible without more experiments to know whether the effect was as great quantitatively as with intact cord. In the one experiment made, β-tetrahydronaphthylamine caused no differential effect. Both with morphine and β-tetra the usual symptoms were elicited, so far as they could be manifested by the portion of the animal innervated from above the cord lesion. In 3 cats, dead or moribund at the time the adrenals were removed, the store was the same in each gland. This has little significance, as the same may be seen with intact

cord, although often in animals dying in the laboratory after section of the nerves of one adrenal a marked difference in the store has been found.

SUMMARY

1. Our previous work on the liberation of epinephrin after the transection of the cervical cord at various levels in acute experiments on cats has been confirmed and extended. The output may be unaltered by the transection, or it may be diminished. Evidence is given that when the output is diminished this is due to "spinal shock" of the mechanism in the thoracic cord concerned in sustaining the epinephrin output. When the bulb and brain were eliminated by a bloodless method (ligation of the head arteries) the output remained uniformly undiminished.

2. In dogs the epinephrin output in acute experiments was always diminished by transection of the cervical cord, owing, it is suggested, to the greater susceptibility to spinal shock of the epinephrin secretory mechanism. The two monkeys examined showed in this regard the same behavior as the dogs.

3. In survival experiments, the output never equalled the average ordinary output, although it was often substantial. In these experiments it appeared that in dogs the output approached more nearly to that found in animals with intact nervous system than in cats, the opposite of what was seen in the acute experiments, as if the secretory mechanism in dogs, although more easily depressed by the spinal section, recovered to a greater degree in the relatively short periods for which the animals were kept alive (up to 13 days). It is not known whether the better general condition of the dogs than of the cats after the operation is a factor in this recovery.

4. Strychnine increases markedly the epinephrin output after transection of the cervical cord both in acute and survival experiments. The action is central (on the thoracic cord).

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THE RELATION OF CATALASE TO HEART ACTIVITY

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The catalase content of body fluids and solid tissues, particularly the relation of catalase to activity and oxidation processes in general, has been the subject of much investigation in recent years, especially by Burge and his coworkers (1). Their results have been striking in many respects although their methods have been criticised by Becht (2) and also by Reimann and Becker (3).

The results which are obtained in attempting to make quantitative determinations of catalase strongly support Becht's statement that no satisfactory method has yet been devised for the determination of catalase in solid tissue; that is, a method giving uniform and accurate measurements of catalase. It is of course impossible to comminute solid tissue, such as heart muscle, to the same degree of separation twice in succession; but such comminutions may be made to approach each other very closely at least, and, provided the determinations are carried out at the same temperature—and this has seemed to be the more decisive factor—fairly accurate and satisfactory results can be secured.

It is also true, as Becht pointed out, that the oxygen release by the catalase of solid tissue acting upon peroxide is not complete in ten minutes (the time period most frequently used as a standard), but it seems proper to assume a quantitative relationship between the amount of catalase present and the amount of gas evolved in a stated period of time; more especially would such a relationship be properly assumed when it is desired to consider catalase in its relation to activity.

The experiments outlined below were begun before the criticisms were offered and previous to the suggestion of more exact methods by Harvey (4); the method used by Burge for the determination of catalase has been followed although the apparatus for the collection of oxygen much more resembled that used by Becht. Since the results

desired were not primarily mere quantitative ones but rather simply a series of measurements which might be properly comparable with each other there would seem to be no valid reason why such comparisons may not be made with the results secured by Burge's method.

The comparative results presented are based upon the amount of gas evolved by 1 gram of finely comminuted ventricular muscle shaken 200 double shakes per minute with 50 cc. of hydrogen peroxide for a period of ten minutes. Proper adjustments for pressure were always made and any results that are compared were always secured at the same temperature, although a uniform temperature has not been maintained for all determinations.

In one of his earlier papers Burge (5) stated that "the amount of catalase in the different muscles of the body varies with the amount of work done by these muscles; those doing the greatest amount of work contain the most catalase, while the muscles doing least work contain least catalase." He stated further that "by increasing or decreasing the external physical work of a muscle the amount of catalase is correspondingly increased or decreased."

Mary Mitchell Moore (6), in presenting evidence for the enzymatic basis of the heart beat, concludes that "the changes in the heart beat of the Fundulus embryo at high temperatures are such as would be expected in case the rhythmical contractions of the heart depend upon the velocity of an enzyme reaction." And further that "since the temperature necessary to bring about standstill of the heart varies with the time of exposure that it indicates a temperature coefficient of the destruction of the enzyme."

From such statements by these two investigators it would seem a proper assumption that the amount of catalase in heart muscle would vary with its activity. To test the truth of such an assumption determinations were made of the catalase present in hearts of turtles (Pseudemys scabra) under varying conditions.

It was found most convenient to make the determinations in sets of threes; the results presented are therefore based upon the use of one control heart and two hearts under modified conditions. Several such group determinations were averaged and whenever the turtle heart was of sufficient size, and this was usually true, two determinations were made with each heart. To cite a typical example of the method of procedure: three turtles were taken and the carapace of each was trephined, exposing the heart. A small opening into the pericardium permitted the introduction of a thermometer and the reading thus

taken has been called "blood" temperature. The trephined opening permitted the heart beat to be counted while at the same time it made simple the subsequent warming or cooling of the entire turtle without loss of blood or other disturbance of the heart. The opening was of sufficient size to allow the rapid removal of the heart at any time it was desired to make a determination of the catalase content.

The following table shows some of the results secured by determinations of catalase:

	BLOOD TEMPERATURE	HEART RATE	TREATMENT	LATER TEMPERATURE	NEW HEART RATE	GAS EVOLVED (AVERAGE)
	°C.			°C.		cc.
A	1	1½	Control			43
	0 to 1	1½	Heart isolated and warmed	28	30 to 40	33
	1	1½	Whole turtle warmed	27	25 to 30	59
B	18	9 to 15	Control			75
	19	16	Heart isolated and warmed	37	70	61
	16	20	Heart isolated and cooled	7	4½	65
C	−2	None; frozen	Turtle slowly warmed 4 hours	14	6	83
	−2	solid	Same; 8 hours	21	21	73

It will be noted that the table has been sub-divided into three parts, A, B and C, representing groups of determinations made under different conditions.

Group A represents turtles that had been kept for several weeks in an ice-covered tank and in which the "blood" temperature was approximately 1°C. and the average heart rate 1½ per minute. The catalase determinations were made at a room temperature of 17°C. Control hearts immediately isolated evolved an average of 43 cc. of oxygen per gram of ventricular muscle in ten minutes. Isolated hearts subsequently warmed until the heart rate was 30 to 40 at a temperature of 28° evolved an average of 33 cc. of gas, a decrease of approximately 25 per cent. Hearts beating 25 to 30 times per minute in situ as the result of warming the entire turtle to 27° showed an increase of about 35 per cent.

In group B the turtles had been kept at room temperature (23°C.) for six hours previous to the determinations with hearts beating at

rates varying from 15 to 20 per minute, one turtle having an arrhythmic rate of 9. Gas evolved by the control hearts averaged 75 cc. in ten minutes; after warming isolated hearts until the heart rate was 70 and heat rigor was imminent at 37°C., the average amount of oxygen was 61 cc., a decrease of 19 per cent, while after cooling the isolated hearts to 7°C., when the heart rate decreased to 4½, the average amount of gas evolved was 65 cc. The difference between the warmed and cooled hearts, or hearts beating 70 times per minute and those beating at 4, is in favor of those at the slower rate—but this is probably to be accounted for by the error of method rather than by any true difference in the catalase content. All determinations in this group were made at 23°C.

Group C is in reality not a "group" as but two turtles were used, although two determinations were made from each. However the results were so striking that they have been included in the table. The turtles had been exposed to a temperature of −6°C. for 15 hours and were frozen solid. After trephining in a warm room the thermometer was forced into the body cavity and registered −2°C. One turtle was slowly warmed for four hours and at the end of that period the heart beat was reëstablished, the rate being 6, reflexes had reappeared, and the intra-pericardial temperature was 14°C. The amount of oxygen released by one gram of ventricular muscle was 83 cc. in ten minutes, being the average of two determinations made at room temperature of 23°C.

The second turtle was permitted to remain at room temperature for an additional four hours, eight hours total, and at the end of that time the intra-pericardial temperature was 21°C. with a heart rate of 21 beats per minute. The amount of gas evolved averaged 73 cc., both determinations giving less oxygen than either of the determinations from the heart beating less than one-third as rapidly.

SUMMARY

Quantitative determinations by Burge's method of catalase in the ventricular muscle of turtle hearts give no evidence that there is any close relationship between the activity of the heart and the amount of catalase present.

Hearts with a rapid rate as the result of warming varied both plus and minus, as compared to controls, in catalase content, the greater number showing less catalase.

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THE EFFECT OF VITAMINE DEFICIENCY ON VARIOUS SPECIES OF ANIMALS

I. The Production of Xerophthalmia in the Rabbit[1]

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It was shown by McCollum (1) and by Osborne and Mendel (2) that the lack of the vitamine or unidentified dietary essential known as fat-soluble A causes a condition of the eyes of rats which is classed as a type of xerophthalmia. The eye-lids become extremely swollen, so that they are opened with difficulty or not at all. The cornea also becomes inflamed, and blindness and death result unless the missing vitamine is supplied before the condition has progressed too far. This specific effect of the lack of fat-soluble A has been observed repeatedly in rats, but consideration of the effect of such deficiency on other species has been generally only by analogy. The occurrence of a condition in children which may have been xerophthalmia was reported from Japan by Mori and from Denmark by Bloch (3). The cases in Denmark were young children who had been fed separator-skimmed milk. Both reported that the patients generally recovered after feeding chicken livers, cod liver oil or whole milk. Cases of eye trouble or blindness in suckling pigs have been occasionally reported to the present authors. The possibility that this may be due to a deficiency of fat-soluble A in the ration of the sows emphasizes further the importance of securing definite information on this subject. The experimental production of this condition in the eyes of rats does not prove, however, that the feeding of a ration deficient in fat-soluble A will produce the same symptoms in another species. The behavior of one species under certain conditions may be exceptional, and instances are on record of the danger of generalization from experiments with a

[1] Published with the permission of the Director of the Iowa Agricultural Experiment Station.

single species. An example is the fact that a diet which produces symptoms of scurvy in a guinea pig does not similarly affect a rat. If the guinea pig requires a certain anti-scorbutic substance, the rat apparently needs a smaller amount or does not need it at all (4).

There are apparently no definite experimental data on the comparative need of different species for fat-soluble A, although it is assumed that all animals need a certain minimum amount of this unidentified substance. It seems possible that the growth impulse may be a factor, or that the dietary habits of the species may influence the relative requirement. Since many herbivorous animals subsist largely on materials rich in fat-soluble A, as green leaves, their relative need for that vitamine may be greater than animals whose natural diet consists largely of seeds and roots. It has been demonstrated that the pig is much more economical with the limited supply of calcium at its disposal than is the dairy cow. This suggests a similar varying requirement for the vitamines also, which are now assumed to be essential factors in the nutrition of all animals.

The study of the requirement of several species of animals for fat-soluble A has been begun by the authors. It is evident from the work of Osborne and Mendel (2) and McCollum (1) that the development of xerophthalmia in the rat is a specific symptom of a marked deficiency in fat-soluble A, and we consider at present that the production of such a symptom in another species is an important step toward determining its relative or absolute requirements for that substance. However, our criterion for the completeness of a ration is not its failure to produce such a definite symptom of malnutrition, but its ability to carry the animal through a normal period of growth and at least one satisfactory reproductive period. Until these points are investigated with other species than the rat the importance of the vitamines in the human dietary, or in the ration of farm animals like the pig, can only be assumed. Furthermore, a deficiency of fat-soluble A in some species may produce some other symptom than xerophthalmia.

EXPERIMENTAL

At the outset of this work a little difficulty was found in securing consumption of a purified ration by the rabbits. After the first week, however, the feed was entirely consumed each day until evidence of malnutrition appeared, coincident with loss of appetite. The ration used was as follows:

Commercial casein	20
Dextrin[2]	65
Salt mixture	5
Lactose	5
Wheat embryo	5

Salt mixture

NaCl	0.45
$MgSO_4.7H_2O$	1.10
$NaH_2PO_4.H_2O$	0.85
K_2HPO_4	2.40
$CaH_4(PO_4)_2.H_2O$	1.35
Ca lactate	3.00
$FeSO_4$	0.25
KI	trace

To this mixture was added alfalfa meal which had been extracted with hot alcohol for three or four days to remove fat-soluble A. Records were kept of the feed consumed each day. The average daily feed per rabbit was 60 grams of the mixture and 20 grams of extracted alfalfa. This was mixed with enough water to moisten it thoroughly. Additional water was allowed.

Three rabbits which averaged about 800 grams in weight were put on this ration, with one litter mate (rabbit 3) as a control on a ration of oats and alfalfa. It might be stated that our controls now receive the purified ration plus butter-fat instead of oats and alfalfa. After a month on the casein-dextrin ration one rabbit, no. 6, was taken out and given a ration of oats, gelatin, dextrin, salts, agar and extracted alfalfa. To date this animal has shown no evidence of xerophthalmia but has remained thrifty and growing. Further data on this animal will be given in a later publication.

Rabbits 4 and 5 remained in a thrifty condition, growing at a normal rate, as compared to the control animal, for a period of sixty-one days. At this point both rabbits began to lose appetite and the eyes became dull and slightly cloudy. A day or two later white films appeared over part of the cornea. At this point butter-fat was mixed with the ration of animal no. 5, but this rabbit refused to eat even fresh green lettuce and became steadily worse. Five days later the eyes were swollen nearly shut and there was considerable whitish exudation. The appearance of the rabbit at this time is shown in figure 1. The next day the animal died, having become entirely blind and having decreased in weight from 2170 grams to 1375 grams in ten days.

Rabbit 4 continued to eat enough to nearly maintain its weight for ten days after the first appearance of an abnormal condition of the eyes.

[2] Starch was moistened with a 0.5–1.0 per cent solution of citric acid and heated in an autoclave at 15 pounds for 1½ hours. The dextrin was broken up while drying, and finally ground.

ERRATUM

Vol. LI, No. 3, pp. 533-534. "The Effect of Vitamine Deficiency on Various Species of Animals. I. The Production of Xerophthalmia in the Rabbit," by Victor E. Nelson and Alvin R. Lamb. The illustrations for figures 1 and 3 are transposed. The legends are correct.

When reading legend of figure 1 cf. cut printed with figure 3; *vice versa*, when reading legend of figure 3, cf. cut printed with figure 1.

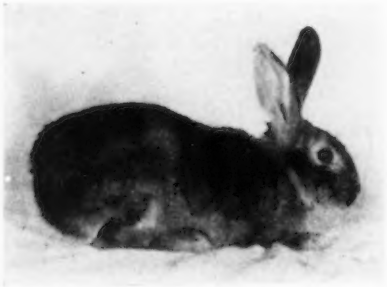

Fig. 1. Rabbit 5, showing xerophthalmia. Weight, 1375 grams.

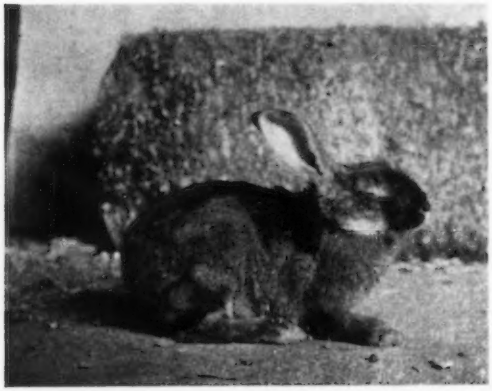

Fig. 2. Rabbit 4, showing eye-lids swollen shut as result of xerophthalmia. Weight, 1430 grams.

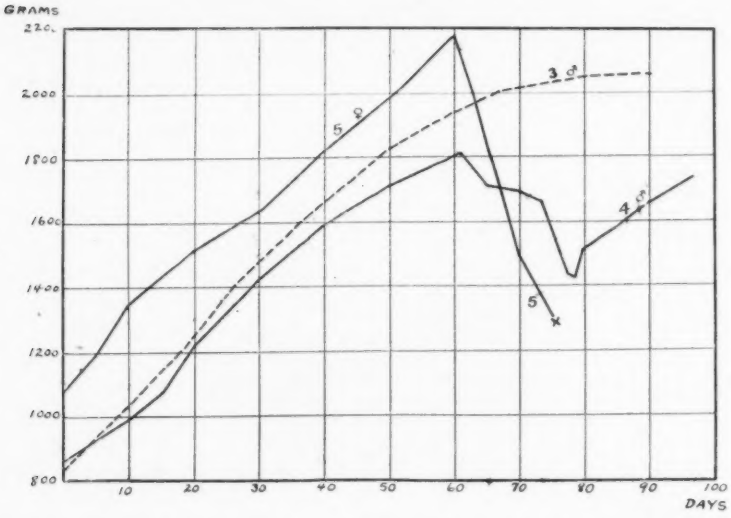

Chart 1. Growth curve of rabbit 3, the control on oats and alfalfa; of no. 5, which died after the onset of xerophthalmia: and of no. 4, which recovered after the administration of butter-fat.

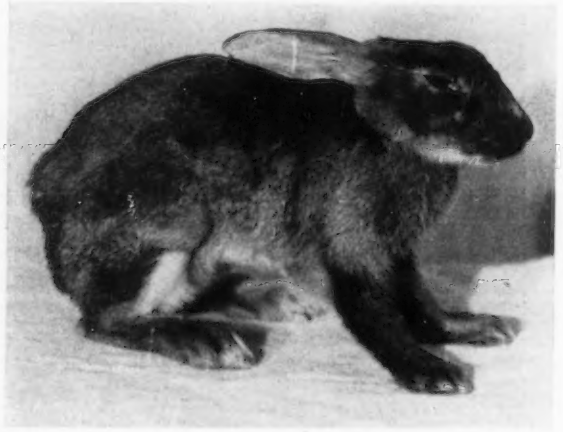

Fig. 3. Rabbit 4, showing recovery of eyes, fifteen days after figure 2. Weight 1652 grams.

At this time, when the eye-lids were swollen nearly shut and were typically congested, the rabbit was photographed (see fig. 2). Ten grams of melted butter-fat were given by mouth with a pipette, and 5 grams each day for four succeeding days were given in the same manner. During these days the regular casein ration was fed and a total of 5 grams of fresh green lettuce and 15 grams of fresh cabbage were given in small amounts to stimulate the appetite. From this point nothing was fed except the regular casein ration to which about 10 per cent butter-fat had been added. In three days after the feeding of butter-fat began the eyes were much improved, and in seven days they were entirely normal in appearance. The animal continued to gain in weight, with increasing appetite and other evidences of well-being, until its loss in weight was entirely recovered (see chart 1). Figure 3 shows the appearance of this rabbit (no. 4) fifteen days after the feeding of butter-fat was begun.

Further experiments on the effect of the lack of fat-soluble A are now in progress, using pigs, guinea pigs, and chickens. We expect to determine the relative requirements of these and other species for this specific vitamine.

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THE FLASHING INTERVAL OF FIREFLIES—ITS TEMPERATURE COEFFICIENT—AN EXPLANATION OF SYNCHRONOUS FLASHING

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For several summers past the writers have had opportunity to observe the flashing of Lampyridae. In the summer of 1915 great numbers (species not identified but probably Photinus pyralis or Photurus) were observed, but only once or twice were the flashings observed to be synchronous. As could be viewed across an open field of about a hundred yards, vast numbers of the insects were flying among the lower branches and at the edge of a wood of oak and chestnut trees. This summer again the phenomenon was observed, but this time quite close at hand in an open lawn, where there could be no possible obstruction to the view. There were a dozen or more beetles flying about three feet above the ground from which they apparently had just risen. The air was perfectly still and very humid. The synchronous character of the flashings continued for six or eight flashes when the insects were lost among some bushes and low trees toward which they had been drifting. The observers were thoroughly convinced then that fireflies, as others before had observed, may at times flash synchronously; and in an effort to gain some light on the cause thereof, the following study was made.

It was first decided to time accurately the interval between flashes of the insects in their normal mating flights. It was soon found that a stop-watch enabling one to read an interval as small as a tenth of a second was a satisfactory instrument. It was soon evident that the temperature was an important factor, and so a good thermometer was hung out in the garden. This practically completed our instrumentarium.

The normal mating flight was chosen for timing the interval between flashes because it is only during this activity that there seems to be

any regularity in the phenomenon. It must have been the experience of others, as it has been ours, that the firefly in captivity will not show any marked regularity in its flashing interval. On the other hand, those individuals that arise in the evening from their day-time haunts and begin to fly about in the air unmolested exhibit a remarkable regularity in the interval between flashes. The deviations in this interval that did occur under such conditions, it seemed to us, were of such an order that they could be easily accounted for by the difference of temperature *a*, of various strata of the air above the earth especially during a calm; *b*, of the bodies of the insects themselves. Those just beginning to fly, and still close to the earth, are probably cooler than those that have been in flight longer. The fact that bees keep themselves warmer in the hive by the activity of their wing muscles would support this assumption. Direct observation of individuals seemed to show some small but constant differences in the flashing interval of insects near their day-time retreats (places moist and cooler than the air itself) and of those higher up in the air, that had been presumably in flight a longer time. The flashing interval of the latter insects was shorter than that of the former.

The duration of the mating flights of the fireflies seemed to be from forty-five minutes to an hour, or about the period of the twilight.

The results of the systematic observations are gathered up in table 1. The individual readings are not recorded but the number of such readings made is stated, their extremes and then their average given together with the temperature of the air. This latter is a mean reading for the evening in each case. For the ten evenings recorded the variations of temperature were respectively 0.4, 0.0, 0.2, 0.75, 0.3, 0.0, 0.2, 1.0, 0.4 and 0.8°C.

The method of making a reading was to record the interval of time between two successive flashes of one individual. It was found impracticable and unnecessary always to follow one and the same beetle for more than one reading. It happened that this could be done sometimes for three or four successive readings and then the interval timed turned out to be the same to the tenth of a second. The intervals for different individuals did not vary greatly for the most part from each other for any one evening, although the extremes, as the table shows, indicate a deviation from 4 to 18 per cent from the mean.[1] In

[1] On the other hand our observations agree with the flashing interval as observed by Mast (1a) for *photinus pyralis* in western Maryland. Curiously, and to our great regret, until now as our own paper is passing through the press we

all the cases where the readings were numerous the average is nearly the same as the mean, so it was thought admissible to incorporate observations for those evenings when the readings were few. For the first two evenings mentioned in the table only the extremes of the observations were recorded, and although the individual observations were numerous, here we have only the mean which is set down in brackets among the averages. Assuming the interval between two successive flashes to be a measure of the rate, this latter was calculated for the minute and set down in the last column of the table. As will be noted this varies from 8 to nearly 16 flashes per minute

TABLE 1

Summary of observations on the normal flashing interval of fireflies at various temperatures

DATE 1919	TEMPERATURE OF AIR	NUMBER OF OBSERVATIONS	FLASHING INTERVAL IN SECONDS		RATE OF FLASHINGS PER MINUTE ($\frac{1}{s}$)
			Extremes	Average interval	
	°C.				
July 25	ca. 28.3	Many	3.5–4.5	[4.0]	15.0
July 26	28.8	Many	3.7–4.1	[3.9]	15.4
July 28	26.0	27	4.2–5.6	4.76	12.6
July 29	22.62	29	5.2–7.0	5.96	10.0
July 31	22.3	30	5.0–7.3	6.03	9.9
August 1	23.2	28	4.9–6.0	5.38	11.1
August 23	24.1	20	4.8–6.0	5.19	11.5
August 24	26.5	11	4.8–5.2	4.93	12.1
August 25	19.4	9	6.5–8.0	7.37	8.1
August 26	17.2	0			

according to the temperature, a rate quite different from that recorded for *Luciola italica*, 80 to 100 by Peters (1), and 60 to 80 by Verworn (2).

It will be noted that as the season advances the number of readings becomes smaller. This is because the insects become fewer. On August 26 not a single firefly was found although we watched for them

have been ignorant of the existence of this excellent article. It should be consulted for its detailed description of the behavior of mating fire-flies.

From Dr. Mast's description it appears that we have probably recorded only the flashing interval of males, since we did not undertake to capture our specimens and identify their sex. The females do not appear to fly during the twilight activity. But since the females flash in reply to the flashing signal only of the male (Mast), one would not anticipate that their interval is greatly different from that of the male.

even longer than usual. The temperature was also the lowest of any of the evenings and probably contributed toward the final disappearance of the firefly for the season.

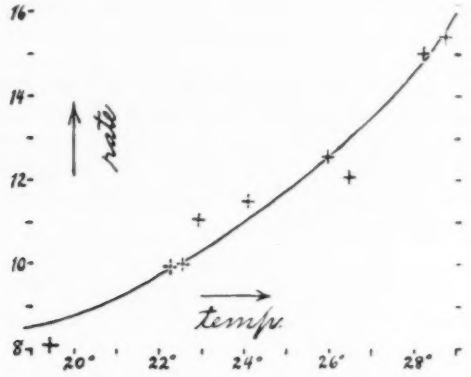

FIG. 1

In the accompanying figure flashing rate is plotted against temperature in which it is seen that the temperature coefficient for a difference of 10°, Q_{10}, is nearly 2, and that the curve is convex to the temperature axis.

In table 2 the flashing rates observed near temperatures 28°, 26°, 22° and 19° respectively are combined and the value of Q_{10} determined from the formula (3)

$$Q_{10} = \left(\frac{k_1}{k}\right)^{\frac{10}{t_1 - t}} \tag{1}$$

from which it appears that the Q_{10} value lies somewhere between 1.99 and 1.95. Let us put it at 1.976 and use this value as a basis for the calculation of flashing rates at the temperatures given in the table for comparison with the observed rates. The magnitude of the temperature coefficient already suggests that the flashing reflex is principally dependent upon chemical reactions. This makes it probable that the flashing rate can be calculated from the van't Hoff formula

$$\log_{10} k = a + bt \tag{2}$$

where k is the observed velocity (in this case the flashing rate) at a given temperature, t, and a and b are constants. These latter values may be easily determined, for the above value of Q_{10} is itself the quotient of two rates at 10 degrees apart and hence

$$Q_{10} = 10^{10.b} \tag{3}$$

Since from (1) $Q_{10} = 1.976$, from (3) $10^{10.b} = 1.976$, whence $b = .0295$. Substituting known values now in equation (2) we find $a = 1.3314$. This enables us to use (2) as often as we like for unknown values of $\log_{10} k$. Such a procedure carried out for the temperatures given in table 2 shows an agreement between the observed and calculated values as complete as one could desire.

TABLE 2

t	k (OBSERVED)	$Q_{10} = 10^{10.b}$	k (Calculated)
28.55	15.2	*1.99*	15.3
26.25	12.35		12.76
22.46	9.99	*1.95* 1.99	10.0
19.4	8.1		8.0

From this we conclude that the interval between the normal mating flashes of the fireflies in our garden[2] (other variables remaining fairly constant) is a function of the temperature; that the interval indicates a rate of flashings varying from 8 to 16 per minute between 19 and 29°C.; and that this variation indicates that, whatever else its nature may be, chemical reactions are the processes fundamental to the flashing mechanism.

The physiologist, however, has already classed the flashing of the Lampyridae among the nervous reflexes of animals. It is known to be independent of the phenomenon of luminescence, a process that is practically continuous or at least pitched at a vastly greater rate. But like the respiratory movements or the heart beat the luminescence is at intervals greatly intensified and this we call the "lightning" or "flashing" of the insects. The sudden irregularities or complete cessation of the flashings observed when the insects are disturbed or put in captivity or when they are at rest or feeding is no more reason to

[2] In view of Dr. Mast's statement (1a) this conclusion can only be made for male specimens for the present. See foot note, page 538.

class the phenomenon as arhythmic than it would be to class thus the flying movements of the bee because they cease when it alights upon a blossom.

Histological studies (4) and histological studies combined with physiological experiments (5) have shown that although not connected directly with the respiratory muscles, as was formerly surmised, the flashings of Lampyridae are nevertheless primarily controlled by "nerves in direct connection with photogenic tissue . . . ." (5). Furthermore, "a direct control relation exists between the photogenic organs and the nerve centers of the head," and most probably there is a direct connection between the optic organs and these centers (6).

That the rate of flashings can not be increased by increasing the rate of an artificial stimulus as has been lately reported by Gates (7), will be easily understood in the light of these remarks and of the results of this study. When the beetles begin their mating flight the flashing mechanism discharges as fast as it can, that is as fast as the temperature permits; and as regularly as it can, that is as regularly as the incident-inhibiting influences permit. If the inhibitions drop to zero the discharges take on a regularity comparable to any other of the well-known rhythmical nervous discharges, such as the respiratory discharge in the medulla of vertebrates, or the discharge in the cord for any of the progression reflexes. But all of these reflexes that have been investigated for the purpose show that they too have a temperature coefficient of the order of chemical reaction velocities (8).

The flashing mechanism of the firefly thus appears not unlike the mechanism of known nerve centers, rhythmically active at times, at others in complete quiescence.

From all this it then appears further that the synchronous flashing of the Lampyridae no longer belongs to the purely accidental nor even necessarily to the extremely rare phenomena of nature. If the extreme deviation from the mean flashing interval is no more than 4 per cent, as it was on one evening of our observations, or even as much as 10 per cent, as it was on another evening (see the data for August 1 and 24 in table 1) it still would be possible for the flashings apparently to occur in unison, that is to occur during the last second, say of an interval of six seconds duration. The necessary conditions for this would include presumably uniformity of moisture, light and air currents as well as temperature.

On the other hand, as far as the individual beetle is concerned, the synchronous flashing would be purely accidental and utterly devoid of

any relation to a higher intelligence or to instinct or any community activity such as one designed to increase the candle-power of a community lighting plant.

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EFFECT OF DIMINISHED OXYGEN UPON RATE OF NERVE CONDUCTION IN CASSIOPEA

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If an annulus of subumbrella tissue of a scyphomedusa such as *Cassiopea xamachana* be stimulated by an induction shock, a contraction wave may be generated which will proceed in one direction through the ring of tissue and continues to course through it at a uniform rate provided the temperature, salinity, CO_2 and other factors of the sea-water remain constant.

Harvey (1) observed that such a contraction wave travelled through a ring of *Cassiopea* at the rate of about 77.5 cm. per second for 11 days. Later Mayor (2) found from a study of 8 rings that the contraction wave moved at an average rate of 44 cm. per second at 28.9°C.; the range among the 8 medusae being from 38.4 to 48.1 cm. per second; and many other unpublished observations made since that time show that about 40 cm. per second is the usual rate in *Cassiopeas* of large size, although each individual has its own characteristic rate, which may vary with age. It will be recalled that Romanes (3) found that the contraction stimulus in *Aurelia* moves at the rate of 22.9 cm. per second.

The question arose as to whether this pulsation was neurogenic, or myogenic or a combination of the two, or whether it was transmitted by the epithelium as well as by the underlying nerve net of the subumbrella of the medusa.

Mayer (4) showed that the stimulus which produced this muscular contraction could pass through recently regenerated tissue which had no muscles and could not contract. Also if a ring of subumbrella tissue be partially immersed in $\frac{5}{8}$ molecular $MgSO_4$ the immersed part soon loses all visible contractibility, yet the stimulus still passes through this non-contracting area and stimulates the normal muscles into contraction. Heating a part of the ring to about 37°C. will produce the same effect, the muscles of the heated area becoming incapable of contrac-

tion and yet the stimulus which has passed through such heated tissue causes contraction in muscles which have been kept at a normal temperature of about 30°C.

Moreover, if the epithelial and nervous elements be peeled off leaving the underlying muscles intact no contraction stimulus can be transmitted through the muscles which are not overlaid by nerves and epithelium.

Bethe (5) shows that the pulsation stimulus in *Rhizostoma* and *Cotylorhiza* readily passes over parts of the subumbrella where no muscles exist. In 1919 at Tortugas, Florida, I scraped areas such as *A*, *B*, *C*

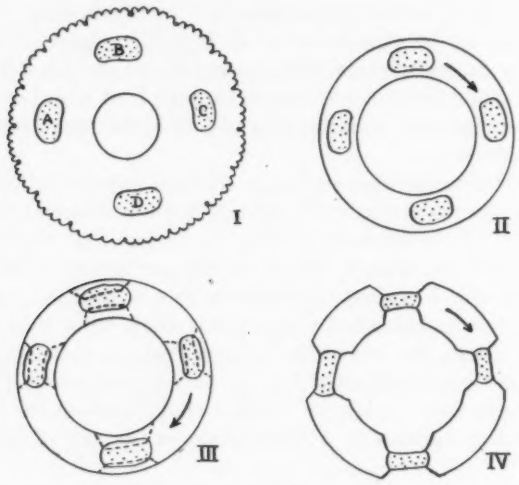

FIG. 1

and *D*, figure 1, (I), so as to remove all muscular, nervous and epithelial structures. Previous experiments had shown that the epithelium and the nerves regenerate before the muscles. Thus at the end of one or two days the scraped area may be healed over by a thin layer of epithelium underlaid by neurons, but the muscles will not usually develop in less than four or five days. If at the end of a day or two we cut an annulus of subumbrella tissue as in figure 1, (II) and activate it with a contraction wave traveling as shown in the arrow we may then make cuts as shown in figure 1, (III), and transform the ring into the shape shown in figure 1, (IV) in which the contraction stimulus

is forced to pass over areas which lack muscles but have nerves and epithelia. In most of the experiments the stimulus could not be made to pass over all of these regenerated areas for one must usually wait some time *after* the epithelium has healed over before the area can transmit the contraction stimulus. This suggests that the stimulus is transmitted by the nerve net and *not* by the epithelium.

In some cases, however, the stimulus was able to pass over the non-contracting regenerated areas and it was thus possible knowing the length of the regenerated areas in comparison with the circumference of the ring to determine the rate of conduction in the regenerated areas by comparing the rate shown when the ring was in the condition illustrated in figure 1, (II) with the rate shown by figure 1, (IV). In various experiments the rate through the regenerating tissue ranged from 11.5 to 46.5 cm. per second. In the case where the regenerating tissue gave a rate of 11.5 cm. the normal tissue of the same ring had a rate of 39.8 cm. per second; but in the example wherein the rate was 46.5 cm. per second in the regenerating tissue, it was 44.6 in the normal tissue of the same ring, the temperature being 30.2°C. Thus the rate of conduction in the regenerating tissue was from about one-third normal, to fully normal in different cases. During the experiment the regenerating tissue was observed under the microscope to see whether it could contract and after the experiment it was killed in Bouin's fluid stained in Ehrlich's haematoxylin and examined. No muscular elements were detected in the regenerating areas in the experiments reported above. Using Hertwig's osmic acid maceration method, Prof. L. R. Cary made preparations of the subumbrella tissue of *Cassiopea*, and these showed bipolar ganglion cells with their fibers forming a loose net-work underlying the epithelium. One could readily imagine that in young regenerating tissue these fibers would be so few in number that the neuron path might not be completed through the tissue in which case no stimulus could be passed through it, or the path might be less direct than when regeneration had gone farther, in which case the apparent rate across the bridge of regenerating tissue would be slower than when more neurons had developed so as to shorten the zig-zags of the path.

Parker (6) stimulated the pedal region of an actinian (*Metridium*), and found that after an interval the oral disc is retracted. By partly cutting off the pedal edge and leaving it as an attached tongue-shaped strip of tissue, and then stimulating the extreme end and afterwards the basal part of the tongue, he found that the rate of the stimulus through the tongue ranged from 12.1 to 14.6 cm. per second at 21°C.

He claims that this experiment is "the only direct determination of the rate of transmission in the nerve net of coelenterates" but his experiment is, I think, open to the objection that he may be dealing with epithelial transmission, or with a combination of nerve-net and epithelial effects, or with tissue poorly provided with neurons thus giving the very slow rate he observed due to a zig-zag path through the neurons. The studies of Wolff (7) and Havet (8) show that the nervous system is poorly developed in the pedal region of Actinians, and as Parker states the pedal region is incapable of contraction.

Parker raises the objection that the rate of muscular contraction in activated rings of *Cassiopea* may not accord with the rate of the stimulus which passes through the neural or epithelial elements. I find, however, (9) that the muscles of the subumbrella rings are capable of

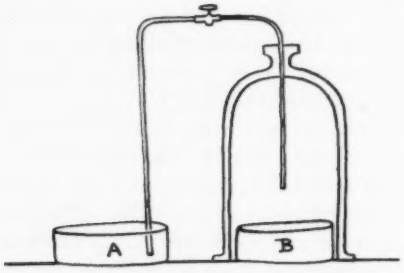

FIG. 2

responding by contractions to stimuli succeeding each other at shorter time intervals than they are called upon to respond to in nature. In other words, in nature they can readily contract to each and every successive recurrence of the stimulus.

It seems certain that in *Cassiopea* the stimulus to which the muscles respond by contraction is neurogenic or epithelial in nature, and the balance of evidence supports the view that it is a neural rather than an epithelial function and we have therefore ventured to call it "nerve conduction."

In 1919 a study was made at Tortugas, Florida, of the relation between the rate of nerve conduction in activated rings of *Cassiopea* and the concentration of oxygen in the sea-water, the oxygen being determined by Winkler's method.

Tortugas sea-water from the surface contains about 4.6 cc. of oxygen per liter of water at 30°C., the oxygen being estimated as at 0°C.

and 760 mm. pressure of mercury. It is thus nearly saturated with oxygen.

The apparatus is shown in figure 2. The activated ring was placed in natural sea-water and its rate determined by counting the number of times per minute the contraction wave traveled around the ring.

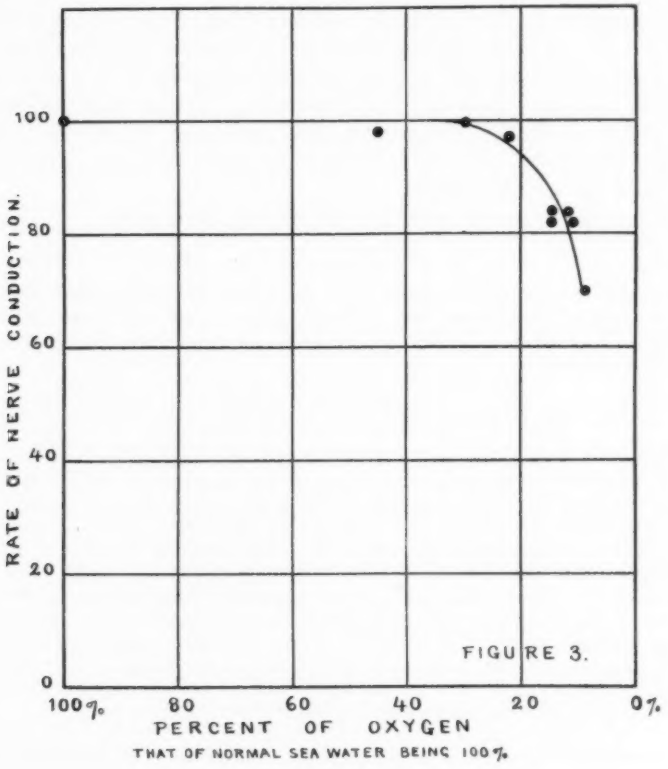

FIG. 3

The temperature of this sea-water was then observed and its oxygen content determined. The pulsating ring was then placed in the glass vessel, *B*, figure 2, under the receiver of an air pump and air was partially exhausted after which sea-water from vessel *A*, was admitted through a fine glass tube. In this manner it was possible quickly to

extract about 96 per cent of the oxygen from the sea-water. When the temperature of the partially exhausted sea-water in vessel *B* had risen to the same temperature as that of the normal sea-water in which the rate had previously been determined, its rate was again ascertained. It was found that after being a few minutes in the partially exhausted sea-water the ring assumed a constant rate which was determined by the degree of concentration of the oxygen in the sea-water. The oxygen in the sea-water in vessel *B* was then determined and compared with that in the natural sea-water in which the rate of the ring had previously been ascertained. These rings appeared to suffer no permanent injury from this treatment for within three minutes after being returned to natural sea-water they were pulsating at normal rate and amplitude.

The results are stated in table 1, illustrated by figure 3.

TABLE 1

Relative rates of nerve conduction in rings of subumbrella tissue of Cassiopea in normal sea-water and in sea-water with gases reduced by placing it under an air pump. The reduction of gases was estimated by determining the oxygen by Winkler's method.

CONDITIONS OF THE EXPERIMENT: RATE OF NERVE CONDUCTION IN NATURAL SEA-WATER CONTAINING ABOUT 4.6 CC. OF OXYGEN PER LITER. (THE OXYGEN IS ESTIMATED AS BEING UNDER 760 MM. PRESSURE, AND AT 0°C.)	RELATIVE RATE OF NERVE CONDUCTION
Rate in natural sea-water	100
Rate in sea-water having 45 per cent of the normal oxygen supply	98
Rate in sea-water having 30 per cent of the normal oxygen supply.	100
Rate in sea-water having 21.9 per cent of the normal oxygen supply	97
Rate in sea-water having 14.3 per cent of the normal oxygen supply	84
Rate in sea-water having 14.3 per cent of the normal oxygen supply	82
Rate in sea-water having 11.9 per cent of the normal oxygen supply	84
Rate in sea-water having 11.5 per cent of the normal oxygen supply	82
Rate in sea-water having 9.3 per cent of the normal oxygen supply	70

The curve derived from these experiments is shown in figure 3.

It seems that the rate of nerve conduction and also the amplitude of the muscular contraction remains practically normal until the oxygen of the sea-water is reduced to about 22 per cent of its normal concentration; but further reduction in the oxygen causes a sharp decline both in rate of nerve conduction and amplitude of the contraction. Thus the reaction accompanying nerve conduction seems to require a concentration of only 1 cc. of oxygen per liter, whereas the concentration of oxygen in normal sea-water is about 4.6 cc. per liter.

Nerve conduction in subumbrella rings must usually maintain at least half its normal rate in order to sustain the ring in pulsation, but the normal *Cassiopea* medusa with marginal sense-organs intact can still pulsate in sea-water containing 4 per cent or even less of the concentration of oxygen found in natural sea-water. Thus the rhopolia can still *engender* the pulsation stimulus and the nerve net can *conduct* it in a concentration of less than 0.18 cc. of oxygen per liter of sea-water.

That nerve conduction is not a simple process of oxidation is shown by the studies of A. V. Hill (10) who demonstrated that there is no appreciable temperature change during the transmission of the nerve impulse. Also Moore (11) shows that there is no increase in the rate of CO_2 production in nerve-muscle preparations of the frog when the nerves are stimulated by a tetanizing current.

However, we know that nerve conduction cannot be wholly independent of oxygen for von Baeyer (12) showed that hydrogen or nitrogen rendered nerves insensible to induction shock, but this sensibility may be restored by oxygen; and moreover Fröhlich (13) showed that the refractory period is prolonged in absence of oxygen.

SUMMARY

In the scyphomedusa *Cassiopea* the pulsation-stimulus is neurogenic.

Nerve conduction remains normal in rate and intensity until the oxygen in the sea-water is reduced to 22 per cent of its normal concentration; thus until there is about 1 cc. of oxygen per 1000 cc. of sea-water, the oxygen being estimated as at 0°C. and 760 mm. pressure of mercury. Further reduction in the concentration of oxygen results in a marked decline in both rate and amplitude of pulsation.

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THE ALKALI RESERVE OF THE BLOOD PLASMA, SPINAL FLUID AND LYMPH

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INTRODUCTION

It has been stated by Van Slyke (1) that the alkali reserve of the blood plasma as measured by its carbon dioxide combining capacity is an index of the alkali reserve of the body fluids. As both lymph and spinal fluid are more or less readily obtained from experimental animals, an investigation to determine primarily the alkali reserve of samples of blood plasma, lymph and spinal fluid taken concurrently, was undertaken. It was early seen that in the case of dogs the carbon dioxide combining power of the spinal fluid varied less in any one animal than that of the blood or thoracic duct lymph, and the investigation was therefore extended with the view of studying changes in the alkali reserve of the spinal fluid, following induced variations in the alkali reserve of the blood.

METHODS

The alkali reserve of blood plasma, lymph and spinal fluid was determined by the method of Van Slyke and Cullen (1).

The blood was aspirated from an artery or vein into a glass syringe containing a trace of powdered potassium oxalate. The oxalated blood was centrifuged within a few moments after withdrawal, and the plasma pipetted off. Lymph was obtained from a cannula placed in the thoracic duct. It was oxalated as collected, to prevent clotting. The spinal fluid was taken as a rule by occipito-atlantoid puncture but occasionally a lumbar puncture was made. The analyses were made within a few minutes after sampling in most instances, but when a delay was necessary the samples were kept in paraffined tubes in an ice chest until the analyses could be made.

In the preliminary experiments venous blood and spinal fluid were taken from dogs while under the influence of morphine. Arterial and venous blood and spinal fluid were taken in several instances from dogs under light ether anesthesia. One series of animals in this latter group received a subcutaneous injection of morphine prior to etherization, while others received ether only. Samples were also taken from dogs under chloroform and alcohol anesthesia, as the protocols indicate.

It is a well-established fact that anesthesia (2) and trauma (3) tend to lower the alkali reserve of the blood.

A number of experiments were performed to determine the effect of prolonged anesthesia and trauma on the alkali reserve of the blood plasma and spinal fluid. Samples of blood and spinal fluid were taken concurrently in these experiments over a period of some hours.

Variations induced in the alkali reserve of the blood plasma and the spinal fluid, as a result of intravenous injections of 5 per cent sodium bicarbonate in distilled water, were followed in another series of experiments. The animals used in this latter series of experiments were in nearly every instance under morphine-ether anesthesia. The samples of blood and spinal fluid were taken concurrently. The readings obtained by the Van Slyke apparatus were reduced to cubic centimeters of CO_2 at 0°C. and 760 mm. bound by 100 cc. of blood plasma, lymph or spinal fluid, at the temperature of 20°C.

We were able to obtain concurrent specimens of spinal fluid and venous blood from a number of human subjects due to the kindness of Doctors Greene and Munroe.

RESULTS

		Alkali Reserve
	Dog 1. 16 kilos. ♂	
4:30 p.m.	Morphine gr. 2; atropine gr. $\frac{1}{100}$ subcutaneous	
6:30 p.m.	Blood aspirated from leg vein	59
6:40 p.m.	Spinal fluid occipito-atlantoid puncture	62
	Dog 2. 14 kilos. ♀	
11:45 a.m.	Morphine gr. 1; atropine gr. $\frac{1}{75}$	
12:00 m.	Spinal fluid lumbar puncture	61
12:10 p.m.	Blood aspirated from femoral vein	52
	Dog 3. 8 kilos. ♀	
4:30 p.m.	Morphine gr. 2; atropine gr. $\frac{1}{150}$	
5:20 p.m.	Chloroform and ether inhalation	
5:30 p.m.	Blood aspirated from leg vein	36
5:35 p.m.	Spinal fluid by occipito-atlantoid puncture	63

Dog 4. 10 kilos. ♀

9:30 a.m.	Ether anesthesia	
10:00 to 1:00 p.m.	Class demonstration	
1:00 p.m.	Blood from femoral vein	27
1:00 p.m.	Spinal fluid by occipito-atlantoid puncture	41

Dog 5. 18 kilos. ♂

10:45 a.m.	Under light ether anesthesia	
11:00 a.m.	Spinal fluid by occipito-atlantoid puncture	54
11:05 a.m.	Blood from femoral vein	49

Dog 6. 14 kilos. ♂

10:15 a.m.	Morphine gr. 3; atropine gr. $\frac{1}{150}$ subcutaneous	
10:40 a.m.	Ether inhalation	
10:50 a.m.	Spinal fluid by occipito-atlantoid puncture	60
11:00 a.m.	Blood from femoral vein	62
11:05 a.m.	Blood from femoral artery	58

Dog 6 (five days later)

10:50 a.m.	Morphine gr. 2; atropine gr. $\frac{1}{75}$ subcutaneous	
11:30 a.m.	Ether	
11:35 a.m.	Spinal fluid occipito-atlantoid puncture	58
11:40 a.m.	Blood from the femoral artery	51

Dog 6 (seven days later)

10:25 a.m.	Under ether	
10:30 a.m.	Spinal fluid occipito-atlantoid puncture	52
10:45 a.m.	Blood from femoral vein	44

Dog 7. 15 kilos. ♂

5:30 p.m.	Morphine gr. 2 subcutaneous	
5:45 p.m.	Ether and chloroform inhalation	
5:50 p.m.	Spinal fluid, occipito-atlantoid puncture	59
5:55 p.m.	Blood from leg vein	55

Dog 8. 12 kilos. ♂

11:00 a.m.	Ether anesthesia	
11:30 a.m.	Spinal fluid, occipito-atlantoid puncture	68
11:35 a.m.	Blood from femoral artery	58

Dog 9. 8 kilos. ♂

10:00 a.m.	Morphine gr. 2; atropine gr. $\frac{1}{150}$	
11:45 a.m.	Ether anesthesia	
11:50 a.m.	Blood from left femoral vein	61
12:00 m.	Spinal fluid, occipito-atlantoid puncture	66
12:30 p.m.	Tracheotomy	

12:25 p.m.	Intestinal massage, and intermittent stimulation of sciatic started	
1:30 p.m.	Blood from the external jugular	50
1:32 p.m.	Blood from femoral artery	46
1:35 p.m.	Spinal fluid	58
2:45 p.m.	Respiratory and cardiac failure, thoracic opened and blood taken from left ventricle	19
2:47 p.m.	Spinal fluid	49

Dog 10. 8 kilos. ♂

8:00 a.m.	Fed pint of milk	
10:20 a.m.	Ether anesthesia	
10:25 a.m.	Spinal fluid	57
10:30 a.m.	Blood from femoral vein	54
10:40 a.m.	Tracheotomy	
11:15 a.m.	Thoracic duct cannularized	
11:25 a.m.	Pancreatic duct cannularized	
12:00 m.	Lymph collected	43
12:15 p.m.	Blood from femoral vein	40
12:10 p.m.	Spinal fluid	53
12:25 p.m.	Lymph	38
12:30 p.m.	Thorax opened	
12:32 p.m.	Blood from left ventricle just before it ceased beating	23

Dog 11. 25 kilos. ♂

12:00 m.	Ether anesthesia	
12:15 p.m.	Spinal fluid	56
12:25 p.m.	Blood from femoral artery	49
12:30 p.m.	Tracheotomy	
1:25 p.m.	Lymph from thoracic duct	47
1:25 p.m.	Blood from femoral artery	47

Dog 12. 14 kilos. ♂

11:00 a.m.	Chloroform anesthesia	
10:00 a.m.	Spinal fluid	52
10:07 a.m.	Blood from femoral vein	39
11:00 a.m.	Thoracic duct cannularized	
11:30 a.m.	Lymph taken	39
12:00 m.	Spinal fluid by occipito-atlantoid puncture	52
12:00 m.	Blood from femoral vein	38
2:30 p.m.	Lymph	32
2:35 p.m.	Spinal fluid	48
2:37 p.m.	Blood from femoral vein	33
4:00 p.m.	Spinal fluid	47
4:08 p.m.	Blood from femoral vein	29

Dog 13. 30 kilos. ♂

10:15 a.m.	Chloroform anesthesia	
10:20 a.m.	Tracheotomy	
10:25 a.m.	Left carotid connected to recorder	
10:30 a.m.	Spinal fluid	63
10:30 a.m.	Blood from femoral vein	39
10:33 a.m.	Started injection of 10 per cent ethyl alcohol in 0.9 per cent saline into left femoral. Chloroform discontinued	
12:30 p.m.	Spinal fluid	53
12:30 p.m.	Blood from femoral vein	32
1:45 p.m.	Spinal fluid	48
1:45 p.m.	Blood from femoral vein, slight hemolysis	37
3:00 p.m.	Spinal fluid	46
3:00 p.m.	Blood from femoral vein	34

Dog 14. 8 kilos. ♀

10:00 a.m.	Ether anesthesia	
10:12 a.m.	Class demonstration (opened thorax)	
12:00 m.	Spinal fluid	42
12:00 m.	Blood from left ventricle	33
12:00 m.	Blood from right ventricle	32

Dog 15. 12 kilos. ♂

3:30 p.m.	Ether anesthesia	
3:40 p.m.	Spinal fluid	51
3:45 p.m.	Blood from femoral vein	45
3:50 p.m.	Intermittent faradization of sciatic	
5:15 p.m.	Blood from femoral vein	35
5:20 p.m.	Spinal fluid	44
6:20 p.m.	Injected 500 cc. of 5 per cent $NaHCO_3$ intravenous	
6:45 p.m.	Blood from femoral vein	115
6:50 p.m.	Spinal fluid	55
8:15 p.m.	Blood from femoral vein	104
8:20 p.m.	Spinal fluid (blood-stained)	82
10:15 p.m.	Blood from femoral vein	101
10:20 p.m.	Spinal fluid (blood-stained)	86
1:00 a.m.	Blood from the femoral vein	84
1:00 a.m.	Spinal fluid (blood-stained)	77
1:10 a.m.	Died	

Dog 16. 35 kilos. ♂

12:30 p.m.	Morphine gr. 3 subcutnaeous	
2:20 p.m.	Ether anesthesia	
2:25 p.m.	Spinal fluid	59
2:25 p.m.	Blood from femoral vein	51

2:40 to 3:20 p.m.	1500 cc. 5 per cent $NaHCO_3$ intravenous	
4:15 p.m.	Blood from femoral vein	141
4:15 p.m.	Spinal fluid	82
5:30 p.m.	Blood from femoral vein	182
5:30 p.m.	Spinal fluid	97
5:35 p.m.	Died	

Dog 17. 15 kilos. ♀

10:45 a.m.	Morphine gr. 2 subcutaneous	
11:15 a.m.	Ether anesthesia	
11:20 a.m.	Spinal fluid	53
11:20 a.m.	Blood from femoral vein	49
11:45 to 12:00 m.	500 cc. 5 per cent $NaHCO_3$ intravenous	
12:20 p.m.	Spinal fluid	62
12:20 p.m.	Blood from femoral vein	123
1:45 p.m.	Died	

Dog 18. 14 kilos. ♂

9:45 a.m.	Morphine gr. 2 subcutaneous	
12:00 m.	Ether anesthesia	
12:10 p.m.	Spinal fluid	55
12:15 p.m.	Blood from femoral vein	50
12:30 p.m.	Tracheotomy. Internal carotid connected to recorder	
1:00 p.m. to 1:15 p.m.	280 cc. of 5 per cent $NaHCO_3$ intravenous	
1:35 p.m.	Spinal fluid	55
1:40 p.m.	Blood from femoral vein	72
4:30 p.m.	Spinal fluid	58
4:35 p.m.	Blood from femoral vein	72
6:10 to 6.25 p.m.	500 cc. of 5 per cent $NaHCO_3$ intravenous	
7:00 p.m.	Spinal fluid	66
7:00 p.m.	Blood from femoral vein	115
10:00 p.m.	Spinal fluid	74
10:00 p.m.	Blood from femoral vein	92
1:10 a.m.	Spinal fluid	80
1:10 a.m.	Blood from femoral vein	102
1:25 a.m.	Died	

Dog 19. 22 kilos. ♂

10:30 a.m.	Ether anesthesia	
10:40 a.m.	Spinal fluid	53
10:40 a.m.	Blood from femoral vein	49
10:55 to 11:15 a.m.	500 cc. of 5 per cent $NaHCO_3$ intravenous	
11:40 a.m.	Blood from femoral vein	104

Dog 20. 12 kilos. ♂

2:00 p.m.	Ether	
2:15 p.m.	Blood from femoral vein	53
2:15 p.m.	Spinal fluid	62

Dog 21. 18 kilos. ♂

11:00 a.m.	Morphine gr. 2	
11:30 a.m.	Etherized	
11:35 a.m.	Blood from femoral vein	40
11:40 a.m.	Spinal fluid	49

Dog 22. ♀

10:45 a.m.	Morphine gr. ½	
11:00 a.m.	Etherized	
11:15 a.m.	Morphine gr. 2. Ether discontinued	
11:30 a.m.	Spinal fluid	51
11:33 a.m.	Blood from femoral vein	45

The CO_2 combining power of specimens of spinal fluid and blood plasma taken concurrently from dogs immediately after anesthesia had been accomplished, is shown in table 1. It will be noted that in all cases but one (dog 6) the CO_2 combining power of the venous plasma is less than that of the corresponding spinal fluid. The difference is quite marked, and varies from 3 to 27 volumes per cent. The CO_2 combining power of the arterial plasma only was determined in dogs 8 and 11. Assuming the CO_2 combining power of the venous plasma to be 4 volumes per cent higher than arterial plasma, there is an average difference of 7.5 volumes per cent in the CO_2 combining power of the venous plasma and the spinal fluid in the twenty-two experiments which have been carried out. The difference is greatest in those instances where chloroform was the anesthetic used, and least where morphine alone or morphine and ether were used.

As the CO_2 combining power of the venous plasma in all the human subjects examined was higher than that of spinal fluid, one hesitates to say that the CO_2 combining power of the spinal fluid of the normal dog is higher than that of the plasma. As Henderson (3) has pointed out, the decrease in the alkali reserve of the blood during anesthesia is primarily due to increased lung ventilation, and therefore to increased rate of CO_2 elimination. It is possible that the blood plasma in practically all our experiments had a lower CO_2 capacity due to increased lung ventilation prior to the time of sampling. There was as a rule more or less panting in dogs shortly after the injection of morphine but when the morphine injection preceded the etherization the latter was accomplished without any marked hyperpnoea being manifested. The alkali reserve of the spinal fluid of the normal animal therefore approximates that of the venous plasma but it is not necessarily identical with

it, while in the human subject the CO_2 combining power of the spinal fluid is definitely lower than that of the plasma.

The differences which are noted in the alkali reserve of the spinal fluid and venous plasma of dogs anesthetized with chloroform compared with those anesthetized with ether are most striking. As there was even less struggle in the experiments where chloroform was used

TABLE 1

CO_2 combining power of plasma and spinal fluid. Concurrent specimens immediately after anesthesia

DOG NUMBER	ANESTHETIC	SPINAL FLUID	VENOUS PLASMA	ARTERIAL PLASMA
1	Morphine only	62	59	
2	Morphine only	61	52	
6	Morphine and ether	60	62	58
6	Morphine and ether 5 days later	58	51	
6	Morphine and ether 2 days later	52	44	
9	Morphine and ether	66	61	
16	Morphine and ether	59	51	
17	Morphine and ether	53	49	
18	Morphine and ether	55	50	
21	Morphine and ether	49	40	
22	Morphine and ether	51	45	
3	Morphine and chloroform	63	36	
7	Morphine, ether and chloroform (trace)	66	61	
12	Chloroform	52	39	
13	Chloroform	63	39	
5	Ether	54	49	
8	Ether	68		58
10	Ether	57	54	
11	Ether	56		49
15	Ether	51	45	
19	Ether	53	49	
20	Ether	62	53	

than when ether was employed, and consequently less hyperpnoea, it would seem that the factor of increased CO_2 loss emphasized by Henderson (3) is not the only one acting to cause a decrease in the alkali reserve of the blood. A possible cause of the greater drop in the CO_2 combining power of the plasma under chloroform than under ether may be found in the difference in the degree of depression of the respiratory exchange in the tissue under these anesthetics. It would be theoretically possible, for example, to have a decrease in the alkali reserve as a

result of decreased CO_2 production, and even decreased CO_2 elimination, providing that the decrease in the former was greater than the decrease in the latter. Thus, as Benjamin Moore (4) has suggested, it may be possible for an actual decrease in the C_H to occur in shock, even though no increased lung ventilation be manifested, due as he expresses it to the respiratory pump working faster than the circulatory pump. If the rate of oxidation, and consequently the rate of CO_2 production in the tissue be decreased, and the lung ventilation remains normal, then a decrease in the combining power of the plasma would occur. Another factor which may be very important is the appearance in the blood of unoxidized acid end products such as aceto-acetic acid and B-oxybutyric as a result of the disturbance of cell metabolism by the anesthetic.

TABLE 2

CO_2 combining power of concurrent samples of spinal fluid, plasma and lymph

DOG NUMBER	SPINAL FLUID	ARTERIAL PLASMA	VENOUS PLASMA	LYMPH
10			40	43
11		47		47
12	48		33	32

The CO_2 combining power of spinal fluid, blood plasma and lymph taken concurrently, is shown in table 2. There is a very close agreement between the values obtained for plasma and lymph in all these experiments. The spinal fluid in dog 12 gave a much higher reading than either blood plasma or lymph. This is in accordance with other experiments of a similar nature. As the alkali reserve of the blood plasma had fallen considerably in dogs 10 and 12 before the samples were taken, it is to be concluded that the alkali reserve of blood plasma and thoracic duct lymph are in close accordance.

The rate of fall in the CO_2 combining power of the blood plasma and spinal fluid with progressive shock due to prolonged anesthesia and trauma is recorded in table 3.

It will be noted in all the experiments quoted that alkali reserve of the spinal fluid never fell to a very low level even when the animal was at the point of death. The alkali reserve of both arterial and venous plasma however fell many points before death ensued. Henderson (5) found that 33 volumes per cent was the critical level for the CO_2 combining power of the plasma. When (as a result of excess lung venti-

TABLE 3

The rate of fall in the rate of CO_2 combining power of plasma and spinal fluid with progressive shock, as a result of prolonged anesthesia and trauma

DOG NUMBER	TIME ELAPSED	SPINAL FLUID	VENOUS PLASMA	ARTERIAL PLASMA
	hours			
9		66	61	
	1½	58	50	46
	2¾	49		19
10		57	54	
	1⅔	53	40	
	2		23	
12		52	39	
	2	52	38	
	4½	48	33	
	6	47	29	
13		63	39	
	2	53	32	
	3¼	48	37	
	4½	46	34	
15		51	45	
	1½	44	35	

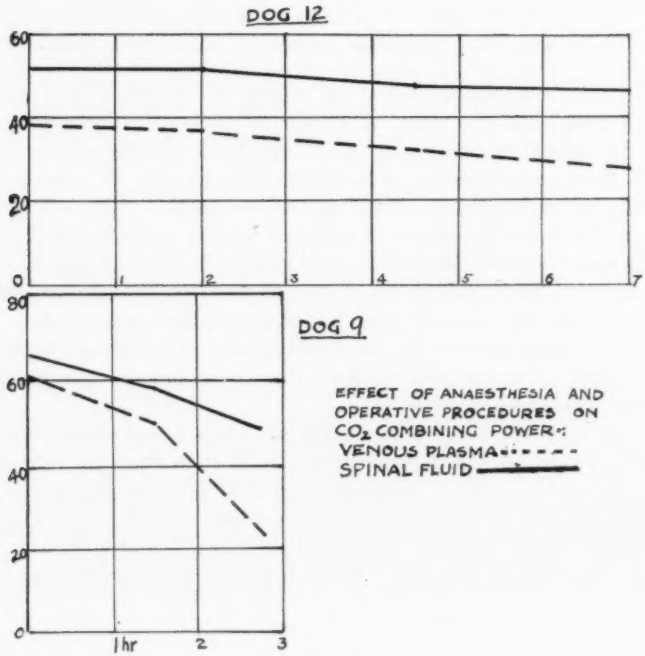

CHART 1

lation) the CO_2 combining power of the blood fell below this level, animals did not recover, while a fall in the CO_2 combining power to a level above 33 volumes per cent did not result in the death of the experimental animal. A steady and progressive fall in the alkali reserve of the plasma occurred in all cases, but in dog 13. (Chart 1, dogs 9 and 12.) In this latter case, the alkali reserve of the blood fell very sharply to 39 in the first few minutes as a result of chloroform

TABLE 4

Rate of rise and fall in CO_2 *combining power of plasma and spinal fluid after* $NaHCO_3$ *injection*

DOG NUMBER	TIME ELAPSED	SPINAL FLUID	VENOUS PLASMA
	hours		
15		44	35
	½	55	115
	2	82	104
	4	86	101
	7	77	84
16		59	51
	1	82	141
	2¼	97	132
17		53	49
	½	62	123
18		55	50
	½	55	72
	3¼	58	72
	2nd injection		
	½	66	115
	3½	74	92
	6¾	80	102

anesthesia and trauma. At the end of two hours it had fallen to 32, while during the next hour and a quarter it rose to 37 and then fell to 34. The plasmas in this experiment showed a trace of hemolysis and this factor may account for the seemingly peculiar behavior of the alkali reserve of the blood in this case.

The fact that the alkali reserve of the spinal fluid remains at a comparatively high level even after shock has been manifested, and indeed even at the point of death, is most significant.

The rate of rise and fall in the CO_2 combining power of the venous plasma and spinal fluid after intravenous injections of $NaHCO_3$ is shown in table 4. The maximum CO_2 combining power of the plasma would of course occur immediately after the bicarbonate injection. In order to give time for equilibrium to be established between the plasma and spinal fluid concurrent samples of these latter were not taken until twenty to sixty minutes had elapsed after the injection was completed.

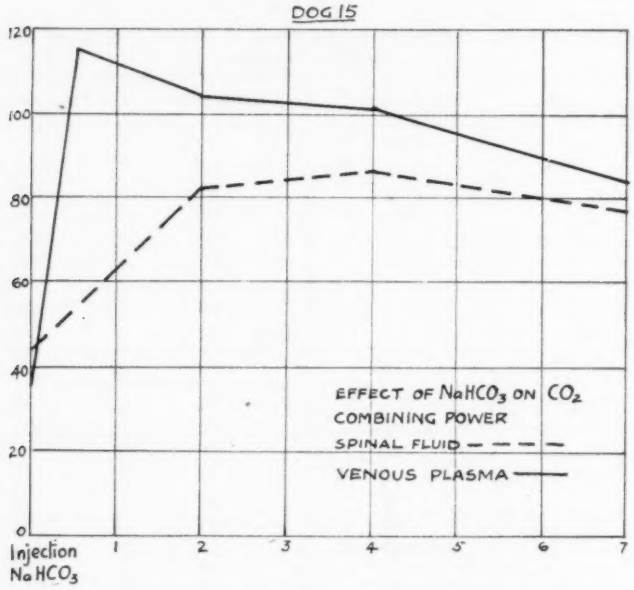

CHART 2

The alkali reserve of the plasma of dog 15 (chart 2) was brought to a low level before the bicarbonate solution was injected. One-half hour after the injection of 500 cc. of 5 per cent $NaHCO_3$ was completed, the CO_2 combining power of the plasma was more than tripled while that of the spinal fluid had risen only 25 per cent or from 44 to 55 as against a rise in the plasma of from 35 to 115. After two hours the plasma bicarbonate had fallen to 104 while that of the spinal fluid was 82. Four hours after the injection the plasma value was 101 while the spinal fluid CO_2 combining power was at its maximum 86 for this experiment.

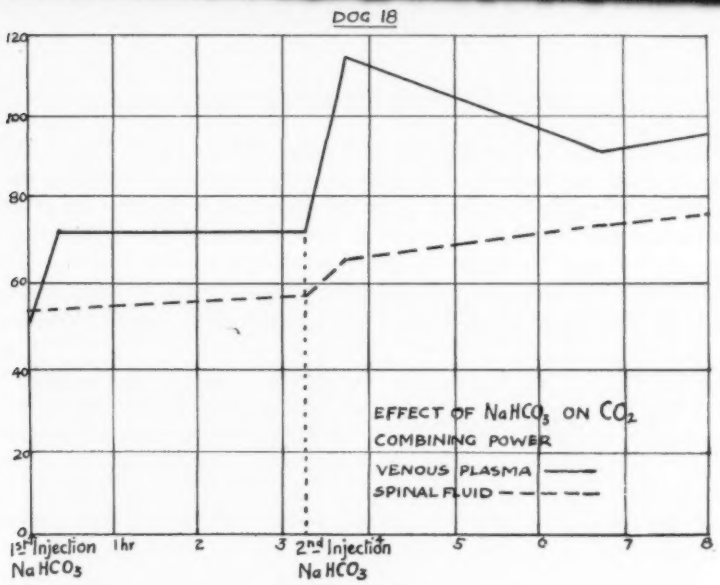

CHART 3

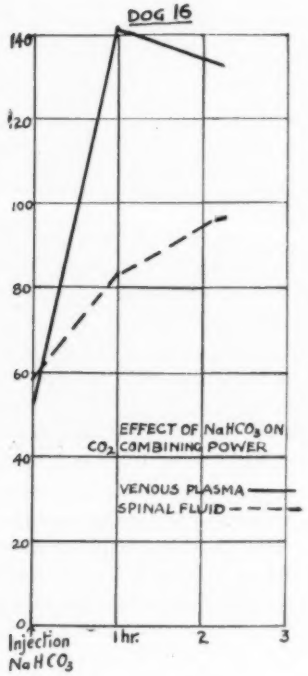

CHART 4

The spinal fluid CO_2 combining power then fell slowly to 77 and that of the blood plasma to 84. The blood pressure was maintained for some hours at a high level. During the last half-hour however it fell rather abruptly and respiratory failure followed soon after. Experiments 16, 17, 18 and 19 (chart 3) show somewhat similar results. The spinal fluid bicarbonate rises very slowly, a great gap between it and that of the plasma being maintained. In dog 18 (chart 4) the alkali reserve of the spinal fluid rose in three and one-fourth hours after the first injection of $NaHCO_3$ from 55 to 58 while the blood plasma reserve was 72 twenty minutes after the injection and this level was maintained for the three hours following. It would appear in this instance that equilibrium had been established between the plasma and the spinal fluid and that an actual difference of fourteen points was being maintained. After the plasma bicarbonate had been raised to a much higher

TABLE 5

CO_2 combining power of venous plasma and spinal fluid in man

SUBJECT	CONDITION	SPINAL FLUID	ASCITIC FLUID	VENOUS PLASMA
1 T. B. T.	Hysteria	48		55
2 T. C.	Hepatic cirrhosis	46	56	58
3 N. H. P.	Normal	60		69
4 R. B. R.	Normal	56		63
5 J. B. A.	Under morphine $\frac{1}{4}$ gr., ether, atropine $\frac{1}{150}$ gr. Osteomyelitis of femur	49		58

level, the spinal fluid bicarbonate value rose slowly reaching a maximum of 80 just before death ensued after six and three-fourths hours.

The respirations were much shallower after the sodium bicarbonate had been injected in those instances where morphine was given subcutaneously prior to anesthetization.

The blood pressure rose slightly after the bicarbonate injection due to the osmotic effect and it was maintained at a high level for very long periods. Death occurred suddenly in all instances, the blood pressure failing first and respiration shortly thereafter (chart 5). Artificial respiration, heart massage and intravenous injection of acid phosphate were without effect. Definite stimulation of respiration both as regards rate and amplitude was obtained in dog 19 during and after the injection of $NaHCO_3$. This animal had received ether only. This would be

in accordance with the observations of Hooker, Wilson, and Connet (6) and Scott (7) that the respiratory center may be stimulated by increased HCO_3 ion concentration more or less independent of the H-ion concentration of the blood.

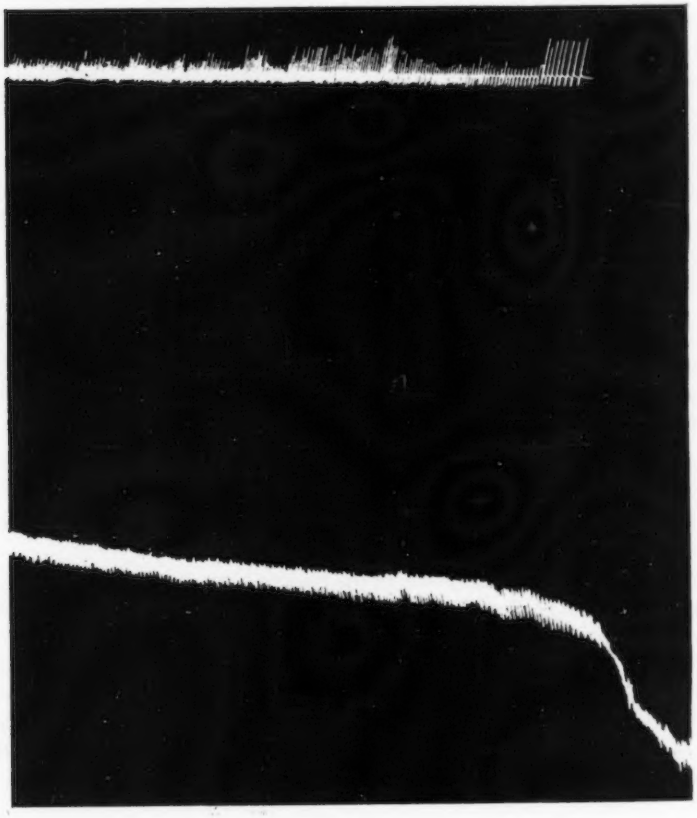

CHART 5

DISCUSSION

The CO_2 combining power of the plasma is not an absolute index of the alkali reserve of all body fluids. The experiments herein reported indicate that there is a very close agreement between the alkali reserve

of blood plasma and lymph. There may also be a very close agreement in the alkali reserve of blood plasma and spinal fluid. The fact, however, that the CO_2 combining power of the spinal fluid does not fall parallel with that of the blood plasma during shock but tends always to maintain a relatively high level, taken in conjunction with the observation that a high level of sodium bicarbonate in the blood does not produce an equally high level for the latter in the spinal fluid, would lend a measure of support to the view of Halliburton (8) that the spinal fluid is the lymph of the brain. It would appear that the tissues of the brain and spinal cord are afforded an extra measure of protection against either an acidosis or an alkalosis of the blood. The C_H of the spinal fluid probably tends to remain constant longer even than the blood during a condition of developing acidosis or alkalosis. Our results may however be interpreted in the light of the theory that the spinal fluid is more or less inert. Sudden changes in the concentration of a blood constituent may not be effective in producing like changes in the concentration of the same constituent in the spinal fluid due to slow or difficult diffusion between the membranes separating the fluid from the plasma. The authors favor the former interpretation of the results of the experiments quoted.

SUMMARY

1. The alkali reserve of concurrent specimens of blood plasma, lymph and spinal fluid has been determined.

2. The CO_2 combining power of the plasma is an approximate index of the "alkali reserve" of the body fluids of the normal animal, as has been claimed by Van Slyke.

3. The CO_2 combining power of the spinal fluid as compared with that of the plasma is maintained at a relatively high level in shock.

4. The CO_2 combining power of the spinal fluid tends to remain at a comparatively low level when the $NaHCO_3$ content of the blood is increased experimentally.

5. It would appear that the C_H of the spinal fluid is protected to a greater degree than that of the blood.

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THE EFFECT OF PROLONGED HYPERPNOEA ON THE CARBON DIOXIDE COMBINING POWER OF THE PLASMA, THE CARBON DIOXIDE TENSION OF ALVEOLAR AIR AND THE EXCRETION OF ACID AND BASIC PHOSPHATE AND AMMONIA BY THE KIDNEY

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INTRODUCTION

It has been shown by Leathes (1) that the amount of acid as compared with basic phosphate in the urine can be materially altered by prolonged hyperpnoea. The method of double titration using two indicators, phenolphthalein and methyl-orange, described by him, furnishes a very simple, yet approximately accurate method whereby one can follow sudden changes in the rate of excretion of total phosphate, and also the relative amounts of the acid and the basic types.

Henderson and Haggard (2) have shown that by increasing or decreasing the rate of carbon dioxide elimination at the lung surface in dogs, the carbon dioxide content as well as capacity of the blood is decreased or increased respectively, the ratio $\frac{CO_2 \text{ dissolved}}{CO_2 \text{ combined}}$ tending to remain a constant. This change in the carbon dioxide combining power of the blood, as Henderson and Haggard (2) have pointed out, indicates that the tissues can either take up alkali from the blood or give up alkali to it, as occasion demands. Henderson, Prince and Haggard (3) found that voluntary hyperpnoea of comparatively short intervals caused a decrease in the venous return to the heart, while systolic, diastolic and pulse pressures were not greatly affected. Henderson (4), Haldane and Poulton (5) and many others have observed marked disturbances of varied character as a result of forced breathing.

The results of a series of experiments carried out upon several members of the medical student body in the University of Alberta, who

kindly offered their services, are reported in this paper. These experiments were designed to follow the changes produced in the carbon dioxide tension of the alveolar air, the carbon dioxide combining power of the venous plasma, the acidity of the urine, and in the rate of excretion of phosphate, ammonia and water by the kidneys, by prolonged voluntary hyperpnoea. A number of observations were also made on blood pressure, pulse rate, hemoglobin and white blood cell content of the blood of these subjects before, during, and after periods of hyperpnoea, while in one instance the percentage of blood sugar was determined before and after a period of forced breathing.

The production of an alkaline urine by a normal individual with a low carbon dioxide combining power of the plasma is of interest. Palmer and Van Slyke (6) have found that in normal men the urine becomes more alkaline than the blood when the CO_2 held as plasma bicarbonate exceeds 71 ± 5 volumes per cent. In prolonged hyperpnoea one finds that the kidney responds in much the same manner as it would if the subject had received a large dose of sodium bicarbonate by mouth or by intravenous injection. The C_H of the blood is the essential factor in the regulation of this phase of renal activity irrespective of what the bicarbonate level may be.

METHODS

Immediately before the initiation of the period of hyperpnoea the subject emptied his bladder, provided a sample of venous blood and a sample of alveolar air. When the period was about to be brought to a close a sample of venous blood and of alveolar air was again taken, the subject emptying the bladder within a few minutes. Further samples of venous blood and alveolar air were taken concurrently fifteen to twenty minutes after, and forty to one hundred and eighty minutes after the cessation of the period of hyperpnoea. The urine in certain cases was taken again as soon as a sufficient quantity had been secreted, usually within an hour. It was also taken in a few cases at intervals of about two hours for a subsequent period.

Blood pressure was taken by the auscultatory method, using either the aneroid type of recorder or the mercury column. Hemoglobin was determined colorimetrically by the acid hematin method, using the Duboscq colorimeter (7). White blood cell counts were made in the usual manner.

The carbon dioxide content of the alveolar air was determined by the method of Fridericia (8) while the CO_2 combining power of the venous plasma was determined by the method of Van Slyke and Cullen (9). The blood was aspirated from an arm vein into a glass syringe containing a trace of powdered potassium oxalate. It was immediately centrifuged and the plasma saturated with carbon dioxide at the alveolar tension of the normal subject. Duplicate analyses were made in practically all instances. The readings were reduced to cubic centimeters of carbon dioxide at 0°C. and 760 mm. bound by 100 cc. of plasma at the respective temperatures.

The ammonia in the urine was determined by the formalin method of Malfatti (10), and calculated in terms of milligrams per 100 cc. of urine and milligrams secreted per hour.

The acidity of the urine was determined according to the titration method of Leathes (1) and represents simply the ratio of acid to basic phosphate.

The amount of N/10 acid, multiplied by 10, required to carry 10 cc. of urine previously made neutral to phenolphthalein to the methyl-orange point, has been taken as an approximate index of the total phosphate, for in such a titration all of the phosphate is first made basic in type and then converted to the acid form. This value is exaggerated slightly in the case of the urine secreted during the stage of hyperpnoea, due to the fact that an appreciable amount of carbonate is present in such a sample. The rate of phosphate elimination per hour was calculated from the data so obtained.

The rate of the secretion of urine was calculated on the basis of cubic centimeters per hour for periods before, during and after hyperpnoea.

Blood sugar was determined by the Lewis Benedict method (11).

The experiments were carried out at different times of the day, the duration of the period of forced breathing varying from fifteen to forty minutes. The results obtained show practically the same types of variations, irrespective of the time the experiments were carried out.

RESULTS

The results of several experiments on various subjects are shown in tabular form in the accompanying tables. Two protocols which are more or less typical of all the others are also shown. A marked diuresis occurred during the period of the experiment in nearly every instance. In the case of J. A. P. where it seemingly did not appear, the sample

voided immediately before the experiment was a four-hour specimen (8:00 a.m. to 12:00 noon), and doubtless secreted in a period during which the morning diuresis would be manifested. The rate of secretion per hour recorded for the period previous to the experiment when such calculation is based on the volume of a sample secreted over a period of some hours is not necessarily therefore a true index of the rate of secretion for the hour immediately preceding the hyperpnoea. The diuresis continued for a few minutes following the cessation of the forced breathing, then the rate of the secretion fell off sharply.

The tension of carbon dioxide in the alveolar air varied from 39.5 mm. in W. A. R. to 50.9 mm. in W. N. S., which represents approximately the range of variation within the normal. After a period of twenty to forty minutes forced breathing the tension of CO_2 in the alveolar air fell to 16 mm. in case of P. E. L. and 28.8 mm. in subject E. S. S. The average decrease in the carbon dioxide tension of the alveolar air in fifteen experiments was 44 per cent.

It was found in those cases in which a determination of the alveolar carbon dioxide tension was made at intervals after the cessation of hyperpnoea, that there was a return to within 4 to 6 mm. of the original tension in from fifteen to twenty minutes, while after longer intervals the alveolar carbon dioxide tension was usually equal to or greater than that existent prior to the experiment. We do not wish to draw any inferences, however, when only small differences in tension are recorded because of the fact that the Fridericia apparatus can only be relied upon to give approximate results.

The carbon dioxide combining power of the venous plasma, taken prior to the period of forced breathing showed about the normal range of variation the average in the fifteen experiments being represented by the factor 66.5. The ratio $\frac{CO_2 \text{ dissolved}}{CO_2 \text{ combined}}$ calculated on the basis of the solubility coefficient of carbon dioxide being 0.52 shows but little deviation from the mean of *0.0470*.

The carbon dioxide combining power of the venous plasma immediately after, or during the last few moments of the period of increased lung ventilation, showed in every instance a marked decrease, the average fall being 14.3 per cent. The sudden return of this factor to the normal value was most striking. This was practically complete in from one to one and a half hours after the hyperpnoea had ceased.

The titratable acidity of the urine fell very abruptly with the onset of hyperpnoea. This was due to the increase in the rate of secretion of

basic, as opposed to acid phosphates, as has been shown by Leathes (1). There was not only a relative increase in the amount of basic phosphates, but there was also an actual increase in the rate of excretion of total phosphates, as well as an actual increase in concentration of the phosphates in the urine. In one instance (E. D. E.) the urine voided immediately after the cessation of hyperpnoea was alkaline to phenolphthalein, 10 cc. requiring 0.05 cc. of $\frac{N}{10}$ acid to make it neutral to this indicator.

The titratable acidity of the urine was low for one to two hours after the experiment, then it rose rather sharply. The total phosphate excretion was lower in the period immediately succeeding the experiment, and the degree of concentration of the same was also less.

Throughout the period of forced breathing the excretion of carbonate was marked but the actual amount secreted was not large.

The concentration of ammonia in the urine fell during the experimental period in all instances. The fall was very marked in some, but less marked in others.

The actual excretion rate of ammonia was always less during hyperpnoea.

The hemoglobin determination, when made, indicated little or no fluctuation in blood volume as a result of the experiment.

The white blood cell counts point to a mild leucocytosis as a result of the forced breathing.

The pulse pressure was decreased as a result of hyperpnoea. This was due to a decreased systolic and an increased diastolic pressure. The change in the character of the pulse, as determined by the palpatory method, was most marked. The actual pulse rate was decreased in some subjects and increased in others.

The blood sugar was found to be increased in one instance where this factor was determined.

Chart 1 illustrates the changes noted in subject E. C. M. as a result of thirty-four minutes hyperpnoea.

The symptoms manifested by the different subjects were for the most part more or less similar, differing only in degree. Objectively the face was flushed in some subjects, it showed little change in others, while in still others both the face and hands became pallid. Muscle tonus was increased in all instances and in a few subjects definite tetany was observed. The muscles affected in these latter instances were those of the fore-arm and hand and to a less extent the muscles of the face and leg. Profuse sweating was noted in a small group of subjects.

Subjectively the individual experienced a tingling sensation very soon after hyperpnoea was commenced. This was localized in the fingers, toes and teeth, and was followed soon after by numbness in the extremities which in a few instances amounted to almost complete cutaneous anesthesia. Most subjects showed a tendency to become drowsy and all were more or less dizzy especially if they attempted to walk. Frontal headache developed in a few subjects. None of our subjects complained of nausea but two remarked that they were unusually hungry and thirsty after their experiment.

PROTOCOLS

J. A. P.

12:00 m. Urine 225 cc. 4-hour sample, 102 mgm. NH_3 per 100 cc. 62 per cent acid.
12:05 p.m. Systolic pressure 112 mm., diastolic 64, pulse 64.
12:15 p.m. Alveolar air, CO_2 = 5.7 per cent
12:17 p.m. Blood from arm vein CO_2 combining power of plasma by Van Slyke method 65
12:17 p.m. to 12:50 p.m. Hyperpnoea
12:50 p.m. Alveolar air CO_2 = 2.8 per cent
12:54 p.m. Blood from arm vein CO_2 of plasma 54
1:00 p.m. Urine 52 cc. 15 mgm, NH_3 per 100 cc. 5 per cent acid
1:05 p.m. Systolic pressure 108, diastolic 80, pulse 70
1:14 p.m. Blood from arm vein, CO_2 of plasma 61
1:15 p.m. Alveolar air, CO_2 = 5.8 per cent
3:15 p.m. Blood from arm vein CO_2 of plasma 65
3:20 p.m. Urine 55 cc., 76 mgm. NH_3 per 100 48 per cent acid

E. C. M.

3:30 p.m. Urine 100 cc. 1½-hour sample, 75 mgm. NH_3 per 100 59 per cent acid
3:35 p.m. Alveolar air, CO_2 = 6.1 per cent
3:40 p.m. Blood from arm vein, CO_2 of plasma = 64
3:10 p.m. to 4:14 p.m. Hyperpnoea
4:10 p.m. Alveolar air, CO_2 = 2.3 per cent
4:13 p.m. Blood from arm vein, CO_2 of plasma = 56
4:15 p.m. Urine 100 cc. 4 mgm. NH_3 per 100 cc. 2.3 per cent acid
4:27 p.m. Blood from arm vein, CO_2 of plasma = 61
5:15 p.m. Urine 50 cc., 29 mgm. NH_3 per 100 6.7 per cent acid
5:25 p.m. Blood from arm vein, CO_2 of plasma = 63
5:27 p.m. Alveolar air, CO_2 = 6.4 per cent
7:05 p.m. Urine 110 cc. 65 mgm. NH_3 per 100 cc. 44 per cent acid
9:00 p.m. Urine 220 cc. 34 mgm. NH_3 per 100 cc. 52 per cent acid
11:35 p.m. Urine 250 cc. 29 mgm. NH_3 per 100 cc. 22 per cent acid

TABLE 1

	J. A. P. 12:00 NOON	E. N. S. 3:30 P.M.	E. S. S. 3:00 P.M.	W. C. C. 4:30 P.M.	E. C. M. 3:30 P.M.	W. A. R. 10:40 A.M.	P. A. L. 4:00 P.M.	G. T. Y. 4:00 P.M.	P. E. L. 3:30 P.M.	P. L. B. 12:45 P.M.	E. D. E. 11:50 A.M.	J. L. J. 4:00 P.M.	C. N. G. 3:10 P.M.	J. G. W. 3:20 P.M.	C. G. R. 11:25 A.M.	P. E. L. 4.30 P.M.	P. E. L 5.00 P.M
Duration of hyperpnoea (minutes)	37	38	35	33	34	32	38	22	32	20	40	33	25	20	23	15	15
Secretion urine, cc. per hour, 1 to 4 hours before	56	50	50	40	66	46	47	144	28	56	35	100	150	90	56		
During hyperpnoea	52	113	84	88	133	220	107	147	100	100	77	172	223	90	90		
After 1 to 2½ hours	24	46	84		50								137				
After 2½ to 6 hours			34	58	83								35				
Per cent of CO_2 in alveolar air 0° and 760 mm. before	5.7	6.7	6.4	6.7	6.1	5.2	6.6	6.4	5.9	5.8	5.7	5.6	5.9	5.6	6.1		
After hyperpnoea	2.8	3.3	3.8	3.2	2.3	3.1	3.1	2.7	2.1	2.7	2.6	2.7	3.0	3.7	2.8		
15 minutes to 20 minutes after	5.8	6.2	5.7	5.9	5.8								5.6				
40 minutes to 80 minutes after			5.9		6.4												
Alveolar CO_2 tension before	43.3	50.9	48.6	50.9	46.3	39.5	50.1	48.6	44.8	44	43.3	42.5	44.8	42.5	46.3		
After	21.3	25	28.8	24.3	17.5	23.6	23.6	20.5	16.0	26.5	20.0	20.5	22.8	28.1	19.3		
CO_2 combining power with plasma before hyperpnoe	65	70	73	69	64	65	65	67	65	65	65	65	65	68	69		
Immediately after	54	62	60	60	56	57	56	51	57	55	59	57	56	56	56		
15 minutes to 20 minutes after	61	67	69	62	61		63										
40 minutes to 180 minutes after	65				63				67						67		
Acidity of urine before hyperpnoea	62	67	32	25	59	23	58	26	65	48	43	32	63	11	44		

Immediately after	5	1.6	3	2.5	2.3	5.5	15	1	3	12	0	3	6	6	10		
After 1 to 2 hours	48	22	8		8								8				
After 2 to 6 hours		76	40	50	44								56				
Ammonia excretion mgm. per hr. before	56	50	22	20	49	15	39	49	40	29	22	52	26	30	19		
During	8	15	10	8	5	13	20	7	25	9	6	24	22	20	13		
After	18	23	17	35	26								31				
Ammonia excretion mgm. per 100 cc. before	102	99	44	51	75	32	82	34	143	52	62	52	17	34	34		
During	15	17	12	9	4	6	22	5	25	9	8	14	10	22	14		
After	76	45	19	60	47								33				
Phosphate excretion per hour before, phenolphthalein to methyl-orange	45	33	20	33	46	21	22	63	21	31	26	50	13.5	93	47		
During	48	141	97	141	173	130	61	150	124	73	127	189	75	133	95		
After	21	30	54	35	37								55				
$\frac{CO_2 \text{ dissolved}}{CO_2 \text{ as bicarbonate}}$ before	0.0456	0.0497	0.0456	0.0504	0.0495	0.0415	0.0527	0.0496	0.0472	0.0465	0.0456	0.0448	0.0472	0.0428	0.0459		
After	0.0268	0.0267	0.0329	0.0277	0.0206	0.0283	0.0288	0.0275	0.0191	0.0255	0.0229	0.0246	0.0279	0.0344	0.0260		
Blood pressure before hyperpnoea S.	112												140		134		
Blood pressure before hyperpnoea D.	68												64		80		
After S.	108												125		130		
After D.	80												82		86		
Pulse before	64												98		82		
Pulse after	70												82		92		
White blood cells before															7,600	8,000	8,500
White blood cells after															10,300	11,200	11,500
Blood sugar before																0.13	
Blood sugar after																0.20	

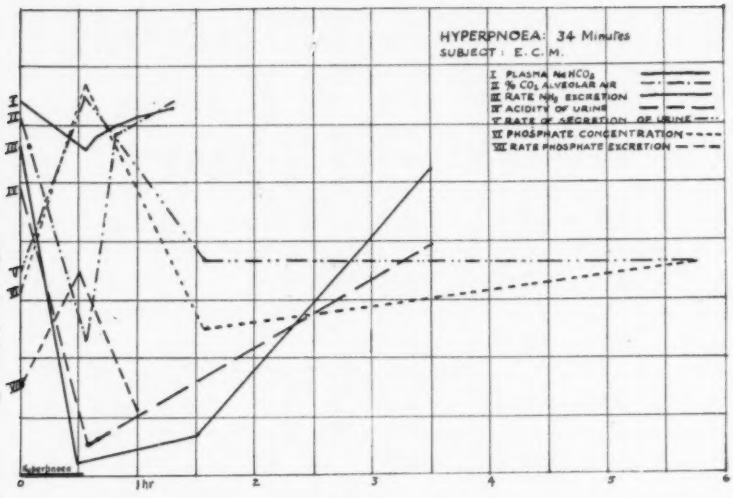

CHART 1

DISCUSSION

The changes produced in the carbon dioxide combining power of the blood and in the rate of excretion of phosphate, ammonia and acid by the kidney, by forced breathing, and the various symptoms noted during hyperpnoea, are interpreted by us as due to the production of a mild degree of alkalosis.

The "washing out of carbon dioxide" from the blood tends to decrease the normal ratio $\frac{H_2CO_3}{NaHCO_3}$ (12), by decreasing the numerator. This would indicate a decrease in the H-ion concentration of the blood. The kidney responds at once by excreting a less acid, or even an alkaline urine (E. D. E.), at the same time the rate of secretion is increased, and a small quantity of sodium bicarbonate is excreted. These facts would favor a mechanical interpretation of kidney function.

Van Slyke (9) has suggested that the alkali reserve of the blood plasma may be taken as an index of the alkali reserve of the whole body. If this were always so it would appear in the experiments reported in this paper that a great decrease in the alkali reserve of the body had occurred. This however is obviously not the case, or the prompt re-

turn of the blood bicarbonate to its normal level within a few minutes after the cessation of hyperpnoea would not be possible.

Again the kidney, while excreting larger quantities of alkali during the experimental periods, does not eliminate sufficient to account for the fall in blood bicarbonate observed. The reaction of the blood is protected to a certain degree during the stage of "washing out of carbon dioxide" by increased excretion of alkali by the kidney. What is most important, however, is the buffer action of the tissue. The tissue soaks up alkali, as Henderson (2) has shown, and in so doing must change its own reaction to an appreciable extent. The alkali which is taken up by the tissue during the stage of low carbon dioxide tension in the blood, is apparently returned to the blood as the carbon dioxide tension of the latter rises following the cessation of hyperpnoea.

Tileston (13) has reported severe tetany in a case of Weil's disease following intravenous injection of sodium bicarbonate solution producing a plasma carbon dioxide combining power of 80 volumes per cent.

We have injected large doses of 5 per cent sodium bicarbonate in distilled water intravenously into dogs under local as well as general anesthesia, but in two cases only has the least sign of tetany been manifested. The dog may however have a greater tolerance for bicarbonate than the human subject.

The ratio $\frac{CO_2 \text{ dissolved}}{CO_2 \text{ combined}}$ which has been determined for all subjects prior to and after the experiment from the data furnished is not in the latter instance a reliable index, as the carbon dioxide actually held in combination as bicarbonate in the venous blood would be several points lower than shown in the table, the plasma in all instances having been saturated with alveolar air from the normal subject. When the true value for CO_2 combined in the venous plasma of the subject at the end of an experiment was determined, it was found that the ratio $\frac{CO_2 \text{ dissolved}}{CO_2 \text{ combined}}$ was still much below normal, thus indicating that an actual decrease in H-ion concentration of the blood had occurred.

Unless there are acids other than carbonic which appear in the blood concomitant with the "washing out of carbon dioxide," the hydrogen ion concentration must be decreased by hyperpnoea. The urine was examined in several instances for aceto-acetic acid but none was found. No examination was made for lactic acid in blood or urine. This latter substance has been shown by Macleod (14) to appear in the urine in increased amount after alkali administration. It is possible that it

might be a compensating factor in these experiments. The decrease in the rate of ammonia elimination observed is in keeping with previous work on the significance of the ammonia factor. We have found that the decrease in the rate of secretion of ammonia is not at all uniform. The excretion is markedly suppressed in some cases, while in others the rate of elimination is only slightly depressed.

The exact cause of the hyperglycemia noted in P. E. L. was not determined.

The leucocytosis noted in some subjects as a result of hyperpnoea is due probably to the mechanical factor involved. The lymph nodes in the thorax are directly stimulated by the excessive respiratory movements and as a result give up white cells. It was found in a control experiment on a dog that no increase in the white cell count resulted after the intravenous injection of $1\frac{1}{4}$ g. of sodium bicarbonate per kilo.

The development of definite tetany and "muscle cramps" in many subjects during hyperpnoea appears to us of great significance. These manifestations are due, it would appear, to an alkalosis of the tissue.

These observations would lend support to the view of Wilson (15) and others that parathyroid tetany is a form of alkalosis.

It is possible that the development of "cramp" in swimmers and also in runners may be due to a tissue alkalosis. The increased lung ventilation, in both swimmers and runners, may result at times in a proportionally greater carbon dioxide loss than production and if such a condition did occur then a tissue alkalosis might result. Ether spasm may also be due to a temporary alkalosis resulting from hyperpnoea.

SUMMARY

1. The effect of prolonged voluntary hyperpnoea in normal adults on the carbon dioxide tension of the alveolar air, the carbon dioxide combining power of the venous plasma, the rate of excretion of water, ammonia, basic and acid phosphates by the kidney has been determined.

2. The average fall in the CO_2 tension of the alveolar air was 44 per cent.

3. The average fall in the CO_2 combining power of the venous plasma was 14.3 per cent.

4. The acidity of the urine was markedly decreased.

5. A diuresis was noticed.

6. The rate of the elimination of phosphates was increased.

7. The rate of ammonia excretion was suppressed.

8. A leucocytosis was observed.
9. Hyperglycemia was produced.
10. Typical symptoms were manifested.
11. It is suggested that muscle "cramp" and ether spasm may be due to a temporary alkalosis.

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IS THE LUMINESCENCE OF CYPRIDINA AN OXIDATION?

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In two papers, published in the January number of this Journal, Kanda (1) has criticised my work (2) on *Cypridina hilgendorfii* and has come to the conclusion that the luminescence of this animal is not an oxidation. Before showing that this is incorrect I wish to discuss several points which Kanda has brought up. While I think it is always unfortunate to engage in controversial communications, the relation of oxygen to luminescence is so fundamental that I feel, in the interest of truth, it is necessary to answer his criticism. I trust that the methods of removing oxygen herein described may be of value to physiologists in general.

First I call attention to the fact that I have dropped the words photogenin and photophelein as used in the paper which Kanda criticises and have adopted Dubois' terminology, luciferase and luciferin, and believe Dubois' interpretation is correct. My reasons for this are given in a paper (3) which clears up many of Kanda's criticisms, published in November, 1918, fourteen months before Kanda's paper appeared and twelve months before Kanda's paper was accepted for publication.

Second, Kanda remarks that I am "misled" in the use of the terms "luminous organ," "luminous gland," etc., and did not realize that the luminous material must be extruded from the gland cell, before the luminescence appears. I have always realized this and on page 320 (2) state that "these" (granules in the gland cells) "are extruded and dissolve to a colorless solution, absolutely free of visible granules, which gives the light." Students of animal light use the word luminous gland whether the luminescence is intracellular or extracellular. Although I am credited with giving a "full account of the maxillary gland" by Kanda I never intended to do this but referred for structural peculiarities to Professor Yatsu's paper (4), which appeared later in the *Journal of Morphology* and the proof of which I corrected. I am

quite aware that two different secretions are formed in the luminous gland. In fact three different types of granules can be observed in the gland of the living animal and Dahlgren (5) has described, from stained sections, four different secretions.

Third, on page 557 (1), referring to protein tests on the luminous material, there is evidently some bad proof reading, but in addition, by not quoting me fully, Kanda gives the impression that since I obtained negative results with protein tests I believe the luminous materials of *Cypridina* are not of protein nature. What I said, that Kanda omits, is, "I do not mean to infer from this" (negative biochemical tests) "that the luminous substance is neither protein, fat, nor carbohydrate but merely that the concentration giving a bright light is too small to respond to chemical tests" (2, p. 322). If Kanda will examine my paper (6) on "The chemical nature of Cypridina luciferin and Cypridina luciferase," which appeared twelve months before his paper, he will find the chemical nature of *Cypridina* luciferin and luciferase fully discussed.

Kanda evidently regards his discovery that the production of light by *Cypridina hilgendorfii* is not an oxidation a most important one, as he devotes a separate paper to this subject (1, p. 561). It would be important if it were true, but it is not true. A very low pressure of oxygen is sufficient to cause luminescence and these traces of oxygen must have been present in Kanda's experiments. Briefly, Kanda's experiment is as follows: He placed dried *Cypridina* powder in a bottle connected with a vacuum pump, a gas generator and a flask of oxygen-free water in such a way that the air in the bottle could be evacuated and then replaced by water saturated with H_2, N_2 or any other gas to be investigated. Rubber stoppers and rubber-glass joints were used. He obtained luminescence when the oxygen-free (supposedly) water is allowed to come in contact with dry *Cypridina* powder in the oxygen-free (supposedly) bottle, and he consequently concluded that no oxygen is necessary for luminescence. This method is faulty in two respects.

First I must point out that it is an exceedingly difficult matter to remove the last traces of oxygen from a gas or from water and to keep the oxygen away, and that it is practically impossible to do this with the cumbersome and complicated apparatus which Kanda uses (1, p. 562). Air will leak in around rubber-glass joints especially when subjected to a vacuum, no matter how tight they may appear. I long ago discarded rubber-glass connections where apparatus must be evacuated and then filled with pure gas. The only way to keep air

from leaking into an apparatus is to have all glass connections or make joints of lead tubing and deKotinsky cement. A small amount of oxygen is present in Kanda's apparatus and this is sufficient to give the luminescence he has observed. I would advise Kanda to test his apparatus by placing a small piece of phosphorus in bottle E and determining if its luminescence ceases under the same conditions in which the luminescence of *Cypridina* continues. The phosphorus must not, of course, be entirely covered with water. The luminescence of phosphorus requires only a very low oxygen pressure and for some time it was thought not to be an oxidation but the earlier investigators did not realize how low is the oxygen pressure necessary for luminescence. It should also be remembered that at ordinary temperatures phosphorus does not luminesce in pure oxygen. Nevertheless the luminescence of phosphorus is the result of an oxidation.

The second source of error is due to the fact that one can never remove all the oxygen from *dried Cypridina* with an air pump. There is some gas present in the dried tissue and if this tissue is moistened with absolutely oxygen-free water, light will result. I cannot attempt to explain how this gas is held in the dried tissue, whether absorbed, chemically bound or mechanically entangled, but it is there. The experiment which has led me to the above conclusion will be found at the end of this paper, after experiments which show the dependence of *Cypridina* luminescence on oxygen have been described.

The following experiment shows conclusively that the luminescence of *Cypridina* is an oxidation and requires the presence of free oxygen. The vessel, *A* (fig. 1), is partly filled through stopcock *a* with a filtered luminescent aqueous solution of luciferin and luciferase. If the luciferin is concentrated and the luciferase dilute, the luminescence will be bright and last with very gradually fading intensity for 20 or more minutes. Tube *B*, which is sealed to *A* with all glass connections through stopcock *b*, contains a sealed-in platinum spiral which can be made to glow by passing an electric current through it. Hydrogen gas from a cylinder is passed over the glowing spiral and through the luminescent solution. Any oxygen which the hydrogen contains is burned to water. This is a much simpler and more efficient way of removing the oxygen than absorption in alkaline pyrogallol. By connecting *a* to an air pump and properly turning the stopcocks *a* and *b*, vessel *A* can be alternately evacuated and filled with hydrogen. Three or four repetitions of this procedure are sufficient to render the luminescent solution in vessel *A* free of oxygen *and the light disappears.*

By turning off the platinum glower, a small amount of oxygen can now be admitted along with the hydrogen, and, allowing this hydrogen, which may contain less than $\frac{1}{2}$ of 1 per cent of oxygen, to bubble

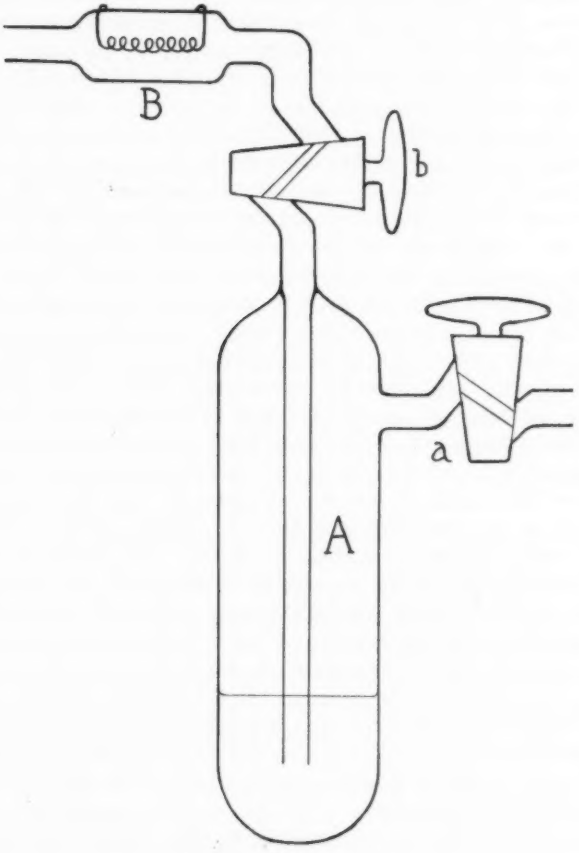

FIG. 1

FIG. 2

through the solution in *A*, *we find that light reappears*. I think this is conclusive evidence that for *Cypridina* luminescence oxygen is necessary and that the photogenic reaction is an oxidation. The same thing can be demonstrated by other methods.

One of the best ways of removing oxygen from a solution is to add living cells to that solution and allow them to withdraw any oxygen that may be present. If a dense suspension of washed yeast cells is added to a glowing mixture of luciferin and luciferase in a test tube, the luminescence, which previously was uniform throughout the tube, very quickly disappears except at the surface in contact with air. If the tube is now stoppered and inverted so as to allow a bubble of air to rise through the solution, the luminescence reappears but again disappears when the oxygen dissolved from this bubble is utilized. The alternate appearance of light on admitting air and disappearance when the yeast uses up all the oxygen can be observed any number of times. The same thing can be shown by allowing *Cypridina* material itself to use up the oxygen. Other phenomena which indicate that *Cypridina* luminescence is an oxidation will be found in my recent paper (7).

Having demonstrated that oxygen is necessary for luminescence I will now describe the experiment which indicates that all the oxygen cannot be removed from dried powdered (in a mortar) *Cypridina* material with an air pump. Kanda assumes that this can be done and I think his principal source of error lies in this assumption. It is known that when water vapor is allowed to freeze at a temperature of solid carbon dioxide, the ice crystals contain no dissolved gases. We may make use of this fact to obtain a small amount of gas-free water. A glass tube, *T*, of the form shown in figure 2, is surrounded at the closed end, *A*, with solid carbon dioxide in a Dewar tube and kept at this low temperature throughout the experimental procedure. A narrow glass tube is then inserted within the larger tube and moist air directed against the cooled surface, *A*. A layer of frost immediately forms. Care must be taken that drops of water do not fall upon surface *A* and immediately freeze as under these conditions bubbles of gas may be mechanically held in the ice. The narrow tube is removed and a little dried *Cypridina* powder is now placed in the bottom of *T*. By inserting again a narrow tube connected with a hydrogen tank, the tube *T* is filled with hydrogen. The *C* end of the tube must be held down in order that the light hydrogen may not escape. Having filled *T* with hydrogen, it is connected to an air pump, thoroughly evacuated and sealed off at *B*. All this time the *A* end of the tube has been kept at a temperature of solid carbon dioxide. The hydrogen used to fill *T* contained 0.43 per cent of oxygen, let us say 0.5 per cent. The vacuum pump was capable of giving a vacuum of less than 0.5 mm. Hg. The partial pressure of oxygen from the gas left in this

tube after evacuation was therefore less than $\frac{1}{200}$ of 0.5 = 0.0025 mm. Hg. We shall return to this figure shortly.

After sealing, tube *T* is removed from the Dewar tube and the ice allowed to melt. As the water surrounds the dried *Cypridina* powder a fair luminescence results. If the tube is now broken and air allowed to rush in, a perfectly brilliant luminescence results. How can this brilliant luminescence be the result of anything else than the oxygen of the air? Since I have previously shown that when the oxygen is completely removed from luciferin and luciferase no luminescence is visible, how can the first weaker luminescence, which is observed when the ice is allowed to melt, be the result of anything but oxygen which does not come out of the tissue in a vacuum, or the oxygen of the residual gas? It must be admitted that 0.0025 mm. Hg. oxygen pressure is a very low one to cause any kind of luminescence and that the light observed must have been the result of oxygen entangled in the tissue rather than the 0.0025 mm. of the residual gas. I find that 0.0025 mm. Hg. oxygen pressure is far too low to cause phosphorus to luminesce. An experiment performed in exactly the same manner as above but substituting a fragment of phosphorus in place of *Cypridina* powder gave no luminescence whatsoever when the ice was melted[1] but a bright luminescence when the tube was broken and air allowed to rush in.

I report this experiment to show that Kanda's method is not suitable and that oxygen comes from somewhere when dried *Cypridinae* are moistened with gas-free water, even under the most favorable conditions for excluding oxygen, and that the oxygen presumably comes from the tissue itself. We may, however, use the tubes prepared as above to again show the dependence of luminescence on oxidation. If such a tube is not opened immediately, the luminescence continues for a while gradually becoming fainter and fainter. With small oxygen pressure the photogenic reaction proceeds very slowly. In the course of 48 hours the light disappears entirely. The luciferin has used up the last traces of oxygen present. If the tube is now broken and air allowed to enter, a brilliant luminescence results. Tubes containing luciferase, luciferin and water may be sealed in absence of oxygen and kept for months without a trace of luminescence, but if air is admitted a brilliant luminescence immediately appears.

[1] It must be pointed out that phosphorus does not require water to luminesce. The water was merely added to carry out the experiment in exactly the same way as the *Cypridina* powder experiment.

Kanda finds that when he admits water in atmospheres of various gases to his dried *Cypridina* material in bottles, E, E_1, the luminescence is at first about equally bright with H_2, N_2, CO, CO_2, air and O_2. Very soon the brightness falls off rapidly in O_2, fairly rapidly in air, but the luminescence continues bright for a long time in H_2 and N_2. We may omit the results with CO and CO_2 since these were found to make the solution acid (1, p. 571) and this introduces another factor. Since the bright light lasts longer, the less oxygen is present Kanda concludes "that oxygen is not necessary for the production of light by the material," and "if the production of light by the animal is due to an oxidation, as Harvey claims, the more intense light should be produced by the greater concentration of oxygen. This is not the case, and the writer therefore concludes that the production of light by the animal is not an oxidation" (1, p. 571).

What has happened in Kanda's experiment is this. The initial amount of oxygen present in bottles, E, E_1, when water is allowed to flow on the *Cypridina* material, is above the amount necessary to give the maximum light. Hence the luminescence is equally bright at first in all gases. In air or oxygen the oxidation goes on sufficiently rapidly to use up the luciferin and reduce its concentration very markedly. Consequently the luminescence fades in these gases. I have found that the intensity of *Cypridina* luminescence is greater the greater the concentration of luciferin, up to a certain limit, and also that the intensity of luminescence, *other factors remaining the same*, does not change when the oxygen concentration is increased from somewhere near 7 per cent to 100 per cent or pure oxygen. In other words, the luminescence intensity is at a maximum when the partial pressure of oxygen is about 53 mm. Hg. (= 7 per cent) and increasing the pressure of oxygen above this value does not increase the intensity of luminescence. The phenomenon is similar to the taking up of oxygen by hemoglobin. At 20°C. hemoglobin is 95 per cent saturated at a partial pressure of oxygen of about 55 mm. Increasing the oxygen pressure above this will not cause much more oxygen to be taken up. As oxyhemoglobin is bright red in color as contrasted with dark red reduced hemoglobin, we may say that it is 95 per cent bright red at 55 mm. pressure of oxygen and will not become an appreciably brighter red if the pressure of oxygen is increased to 760 mm. Hg. (100 per cent or pure oxygen). I have made this comparison with hemoglobin in my book, *The nature of animal light*, now in press and to appear shortly. Many facts bearing on oxidation and luminescence will be found there.

Finally I may add that the experiments here reported were all performed over a year ago, many of them two years ago, but were not published because they demonstrated nothing new. I have recently repeated them to make sure of my facts—and with the same result. I will therefore conclude this article with my statement made in January, 1917 (p. 321), and quoted by Kanda—"Oxygen is necessary for light production as may be seen by placing the crushed animals in an hydrogen atmosphere, or by bubbling hydrogen through a glowing extract of the animals. The light never completely disappears even after a long time, but remains dim so that very little oxygen (as no special precautions were taken to remove the last traces of oxygen from the hydrogen, prepared in a Kipp generator) is sufficient to give light. Upon readmitting oxygen, however, a brilliant glow results. Every other species of luminous animal investigated likewise requires oxygen for luminescence."

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A PHYSIOLOGICAL RESPONSE TO PITUITARY ADMINISTRATION

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Notwithstanding the many investigations that have been carried on along the lines of studying the physiological effects of the administration of pituitary substance, there is yet to be found in the literature any report of the changes induced in the amounts of the total nitrogen, non-protein nitrogenous constituents, sugar and alkaline reserve of human blood as the result of its ingestion.

This paper is a report of such a study made on six males, three patients and three nurses at this hospital, none of whom were endocrinopathic. The bloods were taken from any given individual on the same day of the week and but once a week throughout the period of study. The specimens for analysis were taken three and a half hours after breakfast, it having been found that bloods taken at this time are not significantly different in this respect from bloods taken before the morning meal (1).

The total nitrogen was determined by the micro-Kjeldahl method of Folin and Farmer (2) in a 1 to 50 dilution of 1 cc. of the blood. The non-protein nitrogen, urea nitrogen, creatinine, creatine, uric acid and sugar were determined according to the methods of Folin and Wu (3). The estimations of the amino-acid nitrogen and of the alkaline reserve were carried out with the apparatus and methods of Van Slyke and his collaborators (4), (5). The rest nitrogen is the difference between the sum of the nitrogen found as urea, creatinine, creatine, uric acid and amino-acid and the total non-protein nitrogen. As a matter of interest, the twenty-four hour urea excretion was also determined for the days on which the blood was collected. The period of observation for each subject consisted of nine weeks. During the first three weeks no pituitary was fed. During the second three weeks a 2-grain tablet of desiccated pituitary substance was ingested three times a day.

TABLE 1

Table showing the results of the blood analyses before, during and after the ingestion of desiccated pituitary substance

DATE	PATIENTS											REMARKS
	Total N	NP N	Urea N	Kt N	Kn N	UA N	AA N	Rest N	Sugar	Alkaline reserve	Urea N	
	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.		gm.	
9–15–19		44.1	20.5	0.44	1.35	0.60	5.9	15.3	127	55.52	6.3	Subject 1. Pituitary administered from 10–1–19 to 10–22–19
9–22–19	2.82	38.7	19.8	0.60	1.25	0.83	4.5	11.7	92	69.53	14.6	
9–29–19	3.36	42.3	20.0	0.52	1.44	0.77	5.4	14.2	111	69.12	13.8	
10– 6–19	2.73	37.8	18.7	0.44	1.28	0.93	5.3	11.1	94	72.10	10.8	
10–13–19	3.30	42.9	21.4	0.48	1.54	0.80	5.8	12.9	90	60.25	12.9	
10–20–19	3.33	44.4	24.8	0.48	1.60	1.57	4.2	11.8	115	71.04	10.7	
10–27–19	2.49	40.5	22.2	0.44	1.47	1.10	5.6	9.7	100	65.38	10.7	
11– 3–19	3.00	39.6	20.9	0.48	1.47	1.10	4.5	11.1	100	66.04	10.3	
11–10–19	3.00	48.0	21.4	0.52	1.31	1.03	5.1	18.6	115	71.06	10.9	
9–17–19	3.36	35.7	16.4	0.44	1.31	0.70	4.8	12.0	95	67.88	13.8	Subject 2. Pituitary administered from 10–2–19 to 10–23–19
9–24–19	3.06	37.5	15.5	0.52	1.22	0.73	6.1	13.4	119	52.97	10.9	
10– 1–19	3.90	33.0	12.3	0.52	1.35	0.77	4.0	14.1	125	74.80	8.4	
10– 8–19	2.74	34.5	12.4	0.48	1.38	1.07	6.0	13.2	105	69.10	7.8	
10–15–19	3.00	41.7	19.1	0.48	1.35	1.20	6.7	12.9	123	66.62	8.3	
10–22–19	3.33	40.5	16.2	0.48	1.31	1.60	5.9	15.0	93	67.20	8.7	
10–29–19	3.22	45.3	15.1	0.48	1.31	0.87	6.3	21.2	118	71.32	11.0	
11– 5–19	2.85	38.3	15.8	0.48	1.38	1.33	5.8	14.5	105	71.98	9.3	
11–12–19	3.33	39.3	20.3	0.52	1.44	1.16	5.6	10.3	132	71.07	8.0	
9–19–19	3.19	21.0	14.5	0.41	1.38	0.57	4.5		93	63.17	11.7	Subject 3. Pituitary administered from 10–6–19 to 10–20–19 and from 11–3–19 to 11–10–19
9–26–19	3.06	34.2	11.8	0.44	0.96	0.70	3.7	16.6	137	68.60		
10– 3–19	2.83	31.7	17.0	0.44	1.03	0.60	3.9	8.7	95	68.32	7.2	
10–10–19	3.06	34.8	15.0	0.48	1.41	0.67	4.3	12.9	105	65.77	6.7	
10–17–19	2.91	30.3	15.0	0.44	1.22	0.70	4.7	8.2	95	66.20	10.9	
10–24–19	3.00	30.0	16.9	0.44	1.22	0.63	5.9	4.9	105	68.18	6.1	
10–31–19	3.03	31.2	15.7	0.44	1.35	0.70	5.6	7.4	105	63.48	9.3	
11– 7–19	3.00	31.2	17.2	0.52	1.19	0.73	5.3	6.3	95	65.50	11.8	
11–14–19	3.19	32.4	14.6	0.44	1.25	0.67	4.8	10.6	118	61.84	11.9	

TABLE 2

Table showing the results of the blood analyses before, during and after the ingestion of desiccated pituitary substance

DATE	NORMALS											REMARKS
	Total N	NP N	Urea N	Kt N	Kn N	UA N	AA N	Rest N	Sugar	Alkaline reserve	Urea N	
	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.		gm.	
9–26–19	3.12	39.6	13.2	0.48	1.25	1.16	3.7	19.8	103	69.12	9.0	Subject 4. Pituitary administered from 10–11–19 to 11–1–19
10– 3–19	3.18	35.9	15.3	0.48	1.06	0.67	4.1	14.3	85	63.61	7.9	
10–10–19	4.00	32.4	16.3	0.52	1.12	0.70	3.9	9.9	96	65.38	10.2	
10–17–19	2.80	32.4	15.4	0.44	1.19	0.93	4.5	9.9	101	64.75	9.6	
10–24–19	3.30	30.9	17.7	0.44	1.35	0.93	5.4	5.1	111	71.04	6.5	
10–31–19	3.00	31.2	16.3	0.44	1.35	1.20	5.6	6.3	92	63.95	10.6	
11– 7–19	3.15	34.2	13.0	0.48	1.60	1.07	5.8	12.3	82	71.10	7.6	
11–14–19	3.25	36.6	17.3	0.44	1.82	1.10	4.5	11.4	108	63.42	11.4	
11–21–19	3.06	32.4	16.2	0.44	1.31	1.20	4.5	8.7	119	64.46	10.3	
10–15–19	2.64	35.4	18.4	0.44	1.35	0.90	5.0	9.3	112	63.42	11.0	Subject 5. Pituitary administered from 10–30–19 to 11–20–19
10–22–19	3.00	34.5	17.7	0.48	1.31	1.07	5.1	8.8	104	65.30	13.3	
10–29–19	3.00	36.6	13.9	0.44	1.25	1.27	5.7	14.0	126	63.36	10.6	
11– 5–19	3.00	34.3	17.6	0.48	1.47	1.07	4.5	9.2	115	66.24	10.6	
11–12–19	2.82	30.6	14.1	0.48	1.47	0.87	4.6	9.1	118	63.78	8.1	
11–19–19	3.25	32.7	15.7	0.44	1.35	1.10	4.7	9.4	130	62.53	8.8	
11–26–19	3.00	36.9	17.1	0.44	1.66	0.83	5.6	11.3	138	63.54	10.5	
12– 3–19	2.94	29.0	14.3	0.44	1.38	0.77	5.7	8.4	106	62.55	9.2	
12–10–19	3.30	31.8	12.1	0.48	1.25	0.73	5.1	12.1	113	59.62	15.7	
9–22–19	2.77	33.9	15.1	0.48	0.93	0.80	5.3	11.4	114	61.33	10.0	Subject 6. Pituitary administered from 9–30–19 to 10–20–19
9–29–19	3.52	30.0	14.4	0.48	1.16	0.83	4.1	9.2	133	67.22	9.3	
10– 6–19	2.88	32.1	16.6	0.44	1.16	0.87	4.7	8.3	107	63.57	12.8	
10–13–19	3.15	30.6	16.4	0.44	1.38	0.90	4.5	7.1	96	67.55	6.9	
10–20–19	2.94	35.4	16.4	0.44	1.22	1.27	3.9	12.2	132	67.34	10.9	
10–27–19	2.81	37.3	20.1	0.48	1.44	1.00	4.7	9.6	100	67.24	10.8	
11– 3–19	3.00	37.5	17.8	0.48	1.35	1.40	4.6	11.9	105	61.61	10.8	
11–10–19	3.15	32.1	14.3	0.44	1.16	1.03	5.1	10.1	114	63.39	8.7	
11–17–19	3.30	33.3	16.6	0.44	1.41	0.93	5.3	8.6	98	63.54	9.3	

These tables are the results of the determinations of the various constituents and represent amounts per 100 cc. of blood. The values for the nitrogenous compounds are given in terms of nitrogen. The abbreviations NP-non-protein nitrogen; Kt—creatinine; Kn—creatine; UA—uric acid; AA—amino acid; Alk. Res.; Cc. of CO_2 reduced to 0°; 760 mm. bound as bicarbonate in 100 cc. of plasma. The urea nitrogen expressed in grams are the amounts excreted per 24 hours on the day on which the blood was taken.

During the last three weeks the use of pituitary was discontinued. Any variations from this general plan are noted in the tables.

The only valid and consistent change observed is the increase in the blood uric acid occurring with and following the administration of the pituitary substance. This appeared in four out of the six subjects studied. The concentration of this blood constituent did not fall back to the values observed before the period of pituitary feeding during the three weeks following its administration, which may be taken to indicate either a retarded elimination or destruction of the drug, or that its effect tends to outlast its direct action.

Two explanations of the increase in uric acid concentration in the blood are possible, one being that the ingestion of pituitary substance stimulates the nuclear metabolism resulting in an increased uric acid production, the second being that the drug causes a decrease in kidney permeability. A combination of the two effects may be possible. However since Meyers and Fine (6) have shown that as the kidney permeability is lowered the uric acid excretion seems to be retarded to a greater degree than other soluble nitrogenous blood constituents, and since Addis, Barnett and Shevky (7) have found that the subcutaneous injection of pituitrin is followed by a marked depression of the activity of the kidney in the excretion of urea, it would appear as if the increased uric acid of the blood observed as the concomitant and sequence of pituitary ingestion is due to a decreased permeability of the kidneys.

The two subjects not exhibiting this reaction give the physical and mental characteristics of the adrenal type if we accept the classification of Lavastine (8), and while the interpretation of their non-response is purely speculative it is possible to consider that because of this physiological characteristic the administration of pituitary had but little if any effect upon the kidney permeability. This hypothesis is indirectly supported by the findings of Addis, Shevky and Bevier (9) that "the subcutaneous injections of amounts of adrenalin which increase the urea excreting activity of the kidney and of amounts of pituitrin which depress that activity, have no effect when they are injected together in a certain balanced proportion."

There are no differences in reaction to the drug on the part of the normals as distinguishable from the patients.

SUMMARY

In four out of six individuals studied the ingestion of pituitary substance caused an increase in the uric acid concentration of the blood. This is interpreted as being probably due to a decreased kidney permeability brought about by the administration of the drug.

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